

Wastewater Lab

Week 2

Course #2222



October 3 - 7, 2016

Fleming Training Center



Wastewater Treatment Lab - Week 2

Course #2222

Week 2

October 3 - 7, 2014

Monday, October 3:

8:30 Chlorine Residual

11:00 Lunch

12:15 Testing for Fecal Coliforms and *E. coli*

Tuesday, October 4:

8:30 Nutrients; Ammonia Nitrogen (Sampling and Distillation)

11:00 Lunch

12:15 Ammonia Nitrogen Analyses

2:30 Results of Microbiological Testing

Wednesday, October 5:

8:30 Activated Sludge Process Control; Sensory Observation; Settleometer; Centrifuge Spin

11:00 Lunch

12:15 Oxygen Uptake Rate; Microscopic Examination of Activated Sludge

Thursday, October 6:

8:30 Nutrients: Nitrogen and Phosphorus

11:00 Lunch

12:15 Jar Testing

1:00 Turbidity

2:00 Instrument and Probe Maintenance

3:00 Oil and Grease

Friday, October 7:

8:30 Activated Sludge Math

10:00 Course Review

11:00 Lunch

12:15 Exam and Course Evaluation



Fleming Training Center

2022 Blanton Dr.
Murfreesboro, TN 37129

Phone: 615-898-6506
Fax: 615-898-8064
E-mail: Shannon.Pratt@tn.gov

Wastewater Lab Course - Week 2

Section 1	Total Chlorine.....	page 1
Section 2	Bacteriological.....	page 19
Section 3	Ammonia.....	page 37
Section 4	Activated Sludge Process Control.....	page 55
Section 5	Nutrients.....	page 75
Section 6	Instrument and Probe Maintenance.....	page 103
Section 7	Turbidity.....	page 105
Section 8	Jar Testing.....	page 111
Section 9	Oil and Grease.....	page 117
Section 10	Activated Sludge Math.....	page 121

Section 1 Total Chlorine



TDEC - Fleming Training Center 1

CHLORINE DISINFECTION

Wastewater Laboratory

TDEC - Fleming Training Center 2

Chlorine Uses in WWTP

- Disinfection
- Reduce BOD
- Odor control
- Improve scum and grease removal
- Control activated sludge bulking
- Foam control
- Filter fly control

TDEC - Fleming Training Center 3

Disinfection

- Disinfection is the process that destroys pathogens.
- This is usually through the addition of chlorine.
- Other methods:
 - Heat
 - Bromine
 - Iodine
 - Ozone
 - UV

TDEC - Fleming Training Center 4

Disinfection

- The most important purpose of chlorination of wastewater is disinfection of the plant effluent.
- It minimizes the potential health hazard to humans from waterborne diseases.
- The amount of chlorine necessary to obtain a satisfactory reduction of bacteria will vary greatly with each plant effluent.
- Do not overchlorinate!!!
- Remember the amount of chlorine required will decrease as the quality of your plant effluent improves.

TDEC - Fleming Training Center 5

Odor Control

- Anaerobic conditions will produce hydrogen sulfide with its characteristic rotten egg smell.
- Chlorine can break down hydrogen sulfide as well as other odor producing bacteria.

TDEC - Fleming Training Center 6

Control of Activated Sludge Bulking

- When sludge bulking has been traced to filamentous organisms and the situation doesn't improve by adjusting F:M ratio and nutrient levels, then chlorination may help.
- Chlorination should only be continued until the filamentous population has decreased and a normal bacteria population has established itself.
- Do not overchlorinate, as you could kill the whole process
- The chlorine is added to the return sludge line

TDEC - Fleming Training Center 7

Chlorine

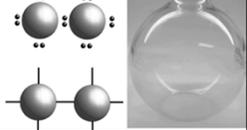
- Gaseous
 - Cl₂
 - 100% pure
- Calcium hypochlorite
 - Ca(OCl)₂
 - 65%
- Sodium hypochlorite (bleach)
 - NaOCl
 - 5.25 – 15%




TDEC - Fleming Training Center 8

Chlorine Properties

- Physical
 - Greenish-yellow, with a penetrating and characteristic odor
 - 2.5 times heavier than air
- Chemical
 - Not corrosive when dry, but very corrosive when mixed with water
 - Chlorine will support combustion and should not be stored near flammable materials
 - Chlorine itself is nonflammable and nonexplosive



TDEC - Fleming Training Center 9

Chlorine Properties



- Toxicity
 - Chlorine gas can be detected by smell by most persons at low concentrations
 - It is a respiratory irritant that will make you cough
 - It can cause irritation to the skin and lungs to a degree that depends upon the concentration and exposure time
 - In sever cases, chlorine gas can cause death from suffocation
 - Liquid chlorine will cause skin "burns" on contact, then it will vaporize and act like chlorine gas
 - Just remember, if you suspect a chlorine gas leak or smell chlorine gas, get out of the area

TDEC - Fleming Training Center 10

Storage of Chlorine

- Do not store in the same room as the dechlorinator (sulfur dioxide, sodium bisulfite, sodium metabisulfite, etc.)
- Exhaust fan intake and exhaust for chlorine storage room located at floor level
- Switches for fans and lights outside cylinder room
- Ammonium hydroxide on hand to test for leaks
- If you do have a leak: 2 in 2 out - required for emergencies; DO NOT BE A HERO

TDEC - Fleming Training Center 11

Affects on pH

- Hypochlorination causes pH to ↑

$$2\text{NaOCl} + 2\text{H}_2\text{O} \rightarrow 2\text{NaOH} + \text{HOCl} + \text{OCl}^- + \text{H}^+$$

$$\text{Ca(OCl)}_2 + \text{H}_2\text{O} \rightarrow \text{HOCl} + \text{Ca(OH)}_2$$
- Gas chlorination causes pH to ↓

$$\text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{HOCl} + \text{HCl}$$

TDEC - Fleming Training Center 12

Chlorine Demand

- The difference between the chlorine added to the water and the amount of residual chlorine remaining after a given time.

$$\text{Demand} = \text{Chlorine Dose} - \text{Chlorine Residual}$$

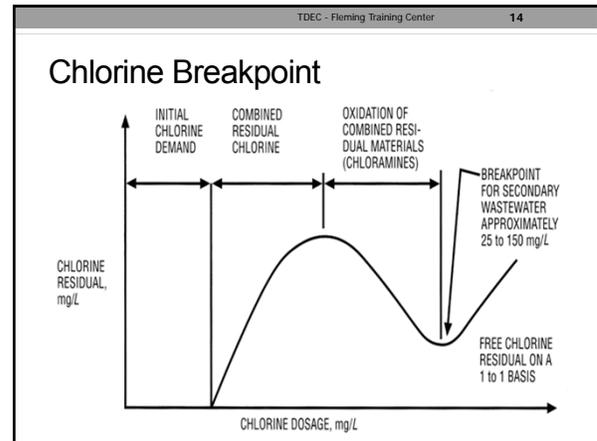
$$\text{Dose} = \text{Chlorine Demand} + \text{Chlorine Residual}$$

TDEC - Fleming Training Center 13

Chlorine Demand

- Wastewater is not pure.
- The reaction of chlorine with impurities interferes with the formation of a free chlorine residual.

Fe ⁺⁺	Inorganic Compounds
Mn ⁺⁺	Nitrogen Compounds
Microorganisms	



TDEC - Fleming Training Center 15

Chlorine Residual Compliance

- Sample NPDES Permit states on page 31 of 32:
 - "This permit contains a residual chlorine limit of 0.02 mg/L based on the instream protection value of 0.019 mg/L for fish and aquatic life. The current limit of detection for residual chlorine is 0.05 mg/L. The Permittee shall obtain the equipment that is necessary to test for residual chlorine down to the detection level. Detection of chlorine in any test will constitute a violation of the permit."

TDEC - Fleming Training Center 16

Chlorine Testing Methods

- Amperometric Titration (back)**
- DPD Colorimetric**
- Starch end-point
- DPD Titrimetric
- Ion Specific Electrode**

***These methods will yield results at the 0.05 mg/L detection level*

TDEC - Fleming Training Center 17

Total vs. Free Chlorine

- Free chlorine refers to both hypochlorous acid (HOCl) and the hypochlorite (OCl⁻) ion or bleach, and is commonly added to water systems for disinfection.
- When ammonia or organic nitrogen is also present, chloramines known as monochloramine, dichloramine, and trichloramine will quickly form.
- Chloramines are also known as combined chlorine.
- Total chlorine is the sum of free chlorine and combined chlorine.

TDEC - Fleming Training Center 18

Total vs. Free Chlorine

- The level of total chlorine will always be higher than or equal to the level of free chlorine.
- Free chlorine is typically measured in drinking water disinfection systems using chlorine gas or sodium hypochlorite to find whether the water system contains enough disinfectant.
 - Typical levels of free chlorine in drinking water are 0.2 - 2.0 mg/L Cl₂, though levels can be as high as 4.0 mg/L.
- Total chlorine is typically measured to determine the total chlorine content of treated wastewater, often for discharge purposes.

DPD Method

- Standard Method 4500-Cl G
- Grab sample, no preservative
- Analyze samples immediately (holding time is 15 minutes)
 - After adding the reagent, a pink color will develop if chlorine is present
 - Wipe the outside of the sample cell with a wet then a dry towel to remove fingerprints

DPD Method - continued

- Hach Procedure:
 - Add DPD to sample and swirl for 20 seconds to mix
 - Wait for a three-minute reaction period
 - Use a timer
 - Within three minutes after timer has ended, read sample

DPD Method - continued

- Interferences
 - Alkalinity > 300 mg/L as CaCO₃
 - Extreme pH: adjust to 6-7 using sulfuric acid or sodium hydroxide (1N)
- Sampling
 - Avoid plastic containers
 - If sampling from a tap, let the water flow at least 5 minutes to ensure a representative sample

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- DOC
- MDL
- LRB
- LFB
- Dup
- ICAL/CCV
- Control Charts
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency



Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Demonstration of Capability (DOC)
 - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
 - No limits listed for chlorine
- Real people language: each operator running this test need to analyze 4 samples of a Chlorine Standard or Potassium Permanganate (KMnO₄) at a concentration around 0.5 mg/L.
- Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
- Recommend backup analyst do this once a year.

Total Residual Chlorine SM4500-Cl G - 2000, DPD

- Method Detection level
 - HACH- Estimated Detection Level=0.02mg/L
 - From SM 1030 C.
 - 0.02mg/L * 5= 0.10 mg/L~ MDL
 - **Make a 0.10 mg/L standard**
 - **Analyze 7 portions over ≥ 3 days**
 - Calculate standard deviation (s)
 - $n1 \Sigma + n2 \Sigma + n3 \Sigma + \dots + n7 \Sigma + 2^{nd} \sigma n = s$
 - $s * 3.14 = MDL$

TDEC - Fleming Training Center 25

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Method Blank
 - Real people language: analyze distilled water as a sample by adding DPD powder pillow and waiting the 3-6 minutes before reading
 - Target value is less than MDL
 - **2014 Update – run on a 5% basis instead of daily**
- Laboratory Fortified Blank
 - Real people language: analyze a chlorine standard or potassium permanganate (KMnO₄) at a concentration around 0.5 mg/L
 - Run on a 5% basis, one for every 20 samples
- Duplicates
 - Run on a 5% basis, one for every 20 samples
 - Calculate %RPD, ≤ 20%
 - **2014 Update – For reporting purposes, average sample and duplicate.**

TDEC - Fleming Training Center 26

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Initial Calibration
 - Prepare a set of chlorine standard or potassium permanganate (KMnO₄) in accordance with the Guidance for Secondary Standards Use in Calibration **monthly**.
 - Once per month at minimum, before the use of new DPD reagents, or the use of new gel standards



TDEC - Fleming Training Center 27

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Stock Standard Solution
 - 0.891 grams of reagent grade KMnO₄ in 1000 mL vol. flask made to mark with deionized water.
 - Deionized water must never be stored in plastic containers or exposed to airborne contamination.
 - Store the stock solution in amber bottle in a cool area.
 - The typical shelf life of the stock solution is six (6) months.
 - If solids appear in the solution, **do not use**.

TDEC - Fleming Training Center 28

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Intermediate (Working) Standard Solution
 - 10 mL of STOCK made in 1000 mL vol. flask made to mark with deionized water.
 - The flask should be labeled with the name, KMnO₄, date of preparation, initials of who made it.
 - This information should also be entered into a logbook.
 - ****The intermediate stock solution should be stable for approximately 5 days if kept cool and away from light.****

TDEC - Fleming Training Center 29

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Potassium Permanganate Standard Solution
 - Care should be taken that the pipette and glassware are clean and thoroughly rinsed with deionized water to avoid contamination.
 - Store only in glass container (preferably amber glass) never in plastic containers.
 - The working solution should be remade if solids appear in the bottom of the container.

TDEC - Fleming Training Center 30

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Calibration Standard Solutions
 - Four to five calibration standard solutions should be made according to the table below to create a calibration curve once per month at a minimum.
 - The linear regression of the curve should correlate to 0.995 or better.
 - This curve is then used to check or calibrate the instrument.
 - Gel standards are run against the curve and must agree to within + 10%.
 - ****The working solution should be stable for approximately 2 hours and will fade rapidly (within 15 minutes) if chlorine demand-free water is not used.****

TDEC - Fleming Training Center 31

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Calibration Standard Solutions
 - A target value (e.g. permit value for a facility) should be known and three gel standards, 0.00 mg/L, blank, and two other standards (a low and a high standard) that bracket the target value should be chosen.
 - Gel standards are run against the curve and must agree to within + 10%.

TDEC - Fleming Training Center 32

Total Residual Chlorine SM4500-CI G - 2000, DPD

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
20 mL (vol. Pipette) to 100 mL (vol. flask)	2.0 mg/L
10 mL (vol. Pipette) to 100 mL (vol. flask)	1.0 mg/L
5 mL (vol. Pipette) to 100 mL (vol. flask)	0.5 mg/L
1 mL (vol. Pipette) to 100 mL (vol. flask)	0.1 mg/L
1 mL (vol. Pipette) to 200 mL (vol. flask)	0.05 mg/L
1 mL (vol. Pipette) to 500 mL (vol. flask)	0.02 mg/L
100 mL of deionized water	0.00 mg/L

TDEC - Fleming Training Center 33

Total Residual Chlorine SM4500-CI G - 2000, DPD

- Calibration Verification
 - Verify meter daily with secondary gel standards using a minimum of one blank and two gel standards that bracket the expected sample concentration



TDEC - Fleming Training Center 34

Total Residual Chlorine SM4500-CI G - 2000, DPD

- **2014 Update** - Create and maintain control charts if you have 20-30 data points within 90 days.
- If you do not meet the above criteria, follow QC Acceptance Criteria below.
 - Blanks < MDL
 - LFB \pm 15%
 - ICV/CCV \pm 10%
 - RPD < 20%
 - Reporting limit = MDL

TDEC - Fleming Training Center 35

Orion Electrode TRC Method

- Model 97-70 chlorine electrode
- Meter – direct reading selective ion or expanded scale pH/millivolt
- Standardization performed with three standards and a reagent blank
- Calculation – concentration determined by direct reading

TDEC - Fleming Training Center 36

Amperometric Titration

- Most sensitive and most complex method
- Least affected by interferences
- Training in proper determination technique
- Titrant initially verified and periodically checked
- Fresh titrant and proper buret
- Titrant storage – dark and cool



TDEC - Fleming Training Center 37

Amperometric Titration

- Apparatus
 - Amperometric Titrator (Wallace & Tieman)
 - Buret with 0.01 mL increments
- Reagents
 - Phenylarsine oxide titrant, 0.00564N
 - Potassium Iodide solution (KI solution)
 - Acetate Buffer solution

TDEC - Fleming Training Center 38

Amperometric Titration - Procedure

- Fill burette with 0.0056N phenylarsine oxide solution (PAO)
- Measure 200 mL of sample into the cell and place in the holder on the titrator
- Add 1 mL Potassium Iodide (KI) solution (5% solution)
- Add 1 mL acetate buffer solution
- Turn on stirrer and adjust control knob until the meter reads the maximum on the scale

TDEC - Fleming Training Center 39

Amperometric Titration - Procedure

- Add phenylarsine oxide in 0.01 mL increments.
 - This should cause the meter reading to deflect downward.
 - Adjust the control knob as needed to keep the pointer on the scale.
 - The end-point is reached when the addition of titrant no longer results in a downward deflection.
- Read the burette, subtracting the amount of the last addition (which did not cause a downward deflection).
 - The burette reading in mL equals the free chlorine residual in mg/L.

TDEC - Fleming Training Center 40

Common Deficiencies

- Sampling and analyses times were not documented for field parameters
- Standards weren't analyzed to verify the accuracy of the chlorine meter
- Measuring free residual chlorine
- Non-approved method being used to measure TRC
- TRC was being measured on the composite sample

TDEC - Fleming Training Center 41

Chlorine Problems

Oh no, not math problems!!



TDEC - Fleming Training Center 42

Chlorine Problems

- A chlorinator is set to feed 50 pounds of chlorine per 24 hours. The wastewater flow rate is 0.85 MGD. The chlorine measured after 30 minutes of contact time is 0.5 mg/L. Find the chlorine dosage and demand in mg/L.

$$\text{Dose, mg/L} = \frac{\text{chlorine lbs/day}}{(Q, \text{MGD})(8.34 \text{ lbs/gal})}$$

$$\text{Dose, mg/L} = \frac{50 \text{ lbs/day}}{(0.85 \text{ MGD})(8.34 \text{ lbs/gal})}$$

$$\text{Dose, mg/L} = 7.1 \text{ mg/L}$$

Chlorine Problems

- A chlorinator is set to feed 50 pounds of chlorine per 24 hours. The wastewater flow rate is 0.85 MGD. The chlorine measured after 30 minutes of contact time is 0.5 mg/L. Find the chlorine dosage and demand in mg/L.

$$\text{Demand, mg/L} = \text{Cl}_2 \text{ Dose, mg/L} - \text{Cl}_2 \text{ Residual, mg/L}$$

$$\text{Demand, mg/L} = 7.1 \text{ mg/L} - 0.5 \text{ mg/L}$$

$$\text{Demand, mg/L} = 6.6 \text{ mg/L}$$

Chlorine Problems

- The chlorine demand is determined to be 5 mg/L and the plant flow rate is 8 MGD. How many pounds per day of gas chlorine should be fed? Include a 1 mg/L residual.

$$\text{Cl}_2, \text{ lbs/day} = (\text{Dose, mg/L})(Q, \text{ MGD})(8.34 \text{ lbs/gal})$$

$$\text{Cl}_2, \text{ lbs/day} = (6 \text{ mg/L})(8 \text{ MGD})(8.34 \text{ lbs/gal})$$

$$\text{Cl}_2, \text{ lbs/day} = 400 \text{ lbs/day}$$

Chlorine Problems

- The chlorine demand is determined to be 5 mg/L and the plant flow rate is 8 MGD. How many pounds per day of HTH (65% chlorine) should be fed? Include a 1 mg/L residual.

$$\text{Cl}_2, \text{ lbs/day} = \frac{(\text{Dose, mg/L})(Q, \text{ MGD})(8.34 \text{ lbs/gal})}{\text{HTH, chlorine percent as decimal}}$$

$$\text{Cl}_2, \text{ lbs/day} = \frac{(6 \text{ mg/L})(8 \text{ MGD})(8.34 \text{ lbs/gal})}{0.65}$$

$$\text{Cl}_2, \text{ lbs/day} = 616 \text{ lbs/day}$$

Chlorine, Total

DOC316.53.01027

USEPA¹ DPD Method²

Method 8167

(0.02 to 2.00 mg/L)

Powder Pillows or AccuVac® Ampuls

Scope and Application: For testing residual chlorine and chloramines in water, wastewater, estuary water and seawater; USEPA-accepted¹ for reporting for drinking and wastewater analyses.

¹ Procedure is equivalent to USEPA method and Standard Method 4500-Cl G for drinking water and wastewater analyses.

² Adapted from *Standard Methods for the Examination of Water and Wastewater*.

! Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

Samples must be analyzed immediately and cannot be preserved for later analysis
If the test overranges, dilute the sample with a known volume of high quality, chlorine demand-free water and repeat the test. Some loss of chlorine may occur due to the dilution. Multiply the result by the dilution factor. Or, analyze samples with high chlorine concentrations directly without dilution by using Method 10070, Chlorine, Total HR.
For chloramination disinfection control, use Method 10172, Chloramine (Mono), Low Range (program number 66) or High Range (program number 67).
After adding reagent a pink color will develop.
The SwiftTest Dispenser ¹ for Total Chlorine can be used in place of the powder pillow in step 3.
An empty AccuVac ampule can be used as a blank in place of the sample cell in step 2.

¹ *Optional reagents and apparatus.*

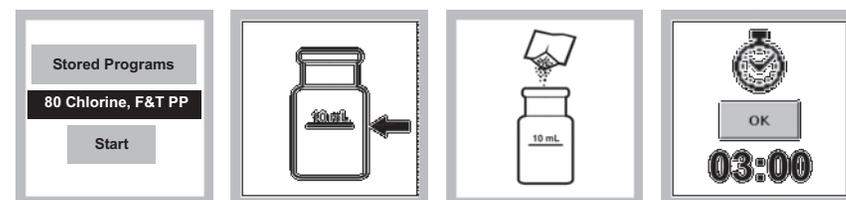
Chlorine, Total

Collect the following items:

Description	Quantity
Powder Pillow Test:	
DPD Total Chlorine Reagent powder pillow, 10-mL	1
Sample Cells (see <i>Instrument-specific information</i>)	2
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
DPD Total Chlorine Reagent AccuVac® Ampul	1
Beaker, 50-mL '	1
Sample Cell (see <i>Instrument-specific information</i>)	1

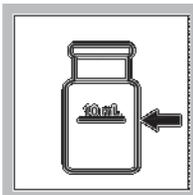
See *Consumables and replacement items* for reorder information.

DPD method for powder pillows



1. Select the test. Insert an adapter if required (see *Instrument-specific information*).
2. Fill a sample cell with 10 mL of sample.
3. **Prepared Sample:** Add the contents of one DPD Total Chlorine Powder Pillow to the sample cell. Swirl the sample cell for 20 seconds to mix.
4. Start the instrument timer. A three-minute reaction period will begin. Perform steps 5 and 6 during this time period.

DPD method for powder pillows (continued)



5. Blank Preparation:
Fill a second sample cell with 10-mL of sample.



6. Wipe the blank sample cell and insert it into the cell holder.

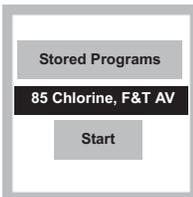
ZERO the instrument. The display will show: 0.00 mg/L Cl₂



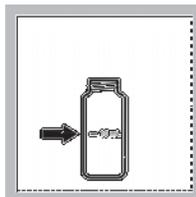
7. Within three minutes after the timer expires, wipe the prepared sample and insert it into the cell holder.

READ the results in mg/L Cl₂.

DPD method for AccuVac Ampuls



1. Select the test. Insert an adapter if required (see [Instrument-specific information](#)). Refer to the user manual for orientation.



2. Blank Preparation:
Fill a sample cell with 10-mL of sample.



3. Prepared Sample:
Fill a DPD Total Chlorine Reagent AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely.



4. Quickly invert the Ampul several times to mix. Wipe off any liquid or fingerprints.

DPD method for AccuVac Ampuls (continued)



5. Start the instrument timer. A three-minute reaction period will begin. Perform steps **6** and **7** during this time period.



6. Wipe the blank sample cell and insert it into the cell holder.

ZERO the instrument. The display will show: 0.00 mg/L Cl₂



7. Within three minutes after the timer expires, wipe the AccuVac Ampul and insert it into the cell holder.

READ the results in mg/L Cl₂.

Interferences

Table 2 Interfering substances and levels

Interfering Substance	Interference Levels and Treatments
Acidity	Greater than 150 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sodium hydroxide ¹ . Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Alkalinity	Greater than 300 mg/L CaCO ₃ . May not develop full color or color may fade instantly. Neutralize to pH 6–7 with 1 N sulfuric acid ¹ . Determine amount to be added on separate sample aliquot, then add the same amount to the sample being tested. Correct for volume addition.
Bromine, Br ₂	Interferes at all levels
Chlorine Dioxide	Interferes at all levels
Chloramines, organic	May interfere
Hardness	No effect at less than 1000 mg/L as CaCO ₃
Iodine, I ₂	Interferes at all levels
Manganese, Oxidized (Mn ⁴⁺ , Mn ⁷⁺) or Chromium, Oxidized (Cr ⁶⁺)	<ol style="list-style-type: none"> Adjust sample pH to 6–7. Add 3 drops potassium iodide¹ (30 g/L) to a 25-mL sample. Mix and wait one minute. Add 3 drops sodium arsenite^{1, 2} (5 g/L) and mix. Analyze 10 mL of the treated sample as described in the procedure. Subtract the result from this test from the original analysis to obtain the correct chlorine concentration.
Ozone	Interferes at all levels
Peroxides	May interfere
Extreme sample pH or Highly buffered samples	Adjust to pH 6–7 using acid (Sulfuric Acid ¹ , 1.000 N) or base (Sodium Hydroxide ¹ , 1.00 N).

¹ See *Optional reagents and apparatus*.

² Samples treated with sodium arsenite for manganese or chromium interferences will be hazardous wastes as regulated by the Federal RCRA for arsenic (D004). Reference the current MSDS for more information on proper disposal of these materials.

Sample collection, preservation and storage

- Analyze samples for chlorine immediately after collection. Chlorine is a strong oxidizing agent and it is unstable in natural waters. It reacts rapidly with various inorganic compounds and more slowly oxidizes organic compounds. Many factors, including reactant concentrations, sunlight, pH, temperature and salinity influence decomposition of chlorine in water.
- Avoid plastic containers since these may have a large chlorine demand.
- Pretreat glass sample containers to remove any chlorine demand by soaking in a dilute bleach solution (1 mL commercial bleach to 1 liter of deionized water) for at least 1 hour. Rinse thoroughly with deionized or distilled water. If sample containers are rinsed thoroughly with deionized or distilled water after use, only occasional pre-treatment is necessary.
- Do not use the same sample cells for free and total chlorine. If trace iodide from the total chlorine reagent is carried over into the free chlorine determination, monochloramine will interfere. It is best to use separate, dedicated sample cells for free and total chlorine determinations.
- A common error in testing for chlorine is not obtaining a representative sample. If sampling from a tap, let the water flow for at least 5 minutes to ensure a representative sample. Let the container overflow with the sample several times, cap the sample containers so there is no headspace (air) above the sample. If sampling with a sample cell, rinse the cell several times with the sample, then carefully fill to the 10-mL mark.
- Perform the chlorine analysis immediately.

Accuracy check

Required for accuracy check:

- Chlorine Voluette® Ampule Standard, 25–30 mg/L Cl₂.
- TenSette Pipet

Standard additions method (sample spike)

- After reading test results, leave the sample cell (unspiked sample) in the instrument.
- Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADDITIONS
DR 2800	OPTIONS>MORE>STANDARD ADDITIONS
DR 2700	OPTIONS>MORE>STANDARD ADDITIONS
DR/2500	OPTIONS>STANDARD ADDITIONS
DR/2400	OPTIONS>STANDARD ADDITIONS

- Default values for standard concentration, sample volume and spike volumes can be accepted or edited. After values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
- Open a LR Chlorine Voluette Ampule Standard, 25–30 mg/L Cl₂.
- Prepare three sample spikes. Fill three mixing cylinders with 10 mL of sample. Use the TenSette® Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively to three 10-mL samples and mix each thoroughly.

Note: For AccuVac® Ampuls, fill three mixing cylinders with 50-mL of sample and spike with 0.4 mL, 0.8 mL and 1.2 mL of standard. Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers*. Analyze each standard addition sample as described in the procedure above. Accept

each standard additions reading by pressing Read. Each addition should reflect approximately 100% recovery.

- Analyze each sample spike as described in the procedure above, starting with the 0.1 mL sample spike. Accept each standard additions reading by pressing Read. Each addition should reflect approximately 100% recovery.
- After completing the sequence, press GRAPH to view the best-fit line through the standard additions data points, accounting for the matrix interferences. Press IDEAL LINE to view the relationship between the sample spikes and the "Ideal Line" of 100% recovery.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
80	DR 5000	1.25 mg/L Cl ₂	1.23–1.27 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR 2800	1.25 mg/L Cl ₂	1.23–1.27 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR 2700	1.25 mg/L Cl ₂	1.23–1.27 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR/2500	1.07 mg/L Cl ₂	1.05–1.09 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR/2400	1.07 mg/L Cl ₂	1.05–1.09 mg/L Cl ₂	0.02 mg/L Cl ₂

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
85	DR 5000	1.25 mg/L Cl ₂	1.21–1.29 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR 2800	1.25 mg/L Cl ₂	1.21–1.29 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR 2700	1.25 mg/L Cl ₂	1.21–1.29 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR/2500	1.07 mg/L Cl ₂	1.03–1.11 mg/L Cl ₂	0.02 mg/L Cl ₂
	DR/2400	1.07 mg/L Cl ₂	1.03–1.11 mg/L Cl ₂	0.02 mg/L Cl ₂

Summary of method

Chlorine can be present in water as free chlorine and as combined chlorine. Both forms can exist in the same water and be determined together as the total chlorine. Free chlorine is present as hypochlorous acid and/or hypochlorite ion. Combined chlorine exists as monochloramine, dichloramine, nitrogen trichloride and other chloro derivatives. The combined chlorine oxidizes iodide in the reagent to iodine. The iodine and free chlorine reacts with DPD (N,N-diethyl-p-phenylenediamine) to form a pink color which is proportional to the total chlorine concentration. To determine the concentration of combined chlorine, run a free chlorine test. Subtract the results of the free chlorine test from the total chlorine test to obtain the combined chlorine concentration. Test results are measured at 530 nm.

Chlorine, Total

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
DPD Total Chlorine Reagent Powder Pillows, 10-mL OR	1	100/pkg	2105669
DPD Total Chlorine Reagent AccuVac® Ampuls	1	25/pkg	2503025

Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
AccuVac snapper	1	each	2405200

Recommended standards

Description	Unit	Catalog number
Chlorine Standard Solution, 2-mL Pour-Rite® Ampule, 25–30 mg/L	20/pkg	2630020
Chlorine Standard Solution, 2-mL PourRite® Ampules, 50–75 mg/L	20/pkg	1426820
Chlorine Standard Solution, 10-mL Voluette® Ampules, 50–75 mg/L	16/pkg	1426810
Voluette Ampule breaker 10 mL	each	2196800
PourRite Ampule breaker 2-mL	each	2484600

Optional reagents and apparatus

Description	Unit	Catalog number
Beakers	50-mL	50041H
Chlorine Demand-Free Water	500 mL	2641549
Cylinder, mixing	25 mL	2088640
Cylinder, mixing	50 mL	189641
Deionized Water	varies	4 L
Potassium Iodide, 30 g/L	100 mL	34332
Sodium Arsenite, 5 g/L	100 mL	104732
Sodium Hydroxide, 1 N	100 mL	104532
Sulfuric Acid, 1 N	100 mL	127032
SwiftTest Dispenser for Total Chlorine	—	2802400
Pipet, TenSette(R), Pipet, 0.1–1.0 mL	each	970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
pH Paper, 0–14 pH range	100/pkg	2601300
AccuVac, vials for sample blanks	25/pkg	2677925
DPD Total Chlorine Reagent Powder Pillows, 10-mL	1000/pkg	2105628
DPD Total Chlorine Reagent Powder Pillows, 10-mL	300/pkg	2105603
DPD Total Chlorine Reagent, 10 mL, SwiftTest Dispenser refill vial	250 tests	2105660
SpecCheck Secondary Standard Kit, Chlorine DPD	0-2.0 mg/L	2635300

Chlorine

Chlorine, Total
Page 375 of 376

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

© Hach Company, 2007. All rights reserved. Printed in the U.S.A.

Updated March 2008, Edition 1

Minimum Detectable Concentration – 4500-Cl G.1.c. – approximately 10 µg/L (0.010 mg/L)

Initial Demonstration of Capability (DOC)

- 4020 B.1.a. - each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – each operator running this test needs to analyze 4 samples of a chlorine or potassium permanganate (KMnO₄) standard at a concentration of 0.5 mg/L**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**
 - **Only good for that type of instrument you are using at that plant. If you have a backup instrument made by a different manufacturer or you work at another plant with a different make/model, you would need another DOC.**
 - **DOCs demonstrate you are proficient with that one instrument.**

Method Detection Limit (MDL)

- 1020 B. 4 – As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level (5 x 0.010 mg/L = 0.050 mg/L).
 - Ideally, prepare and analyze at least seven (7) portions of this solution over a 3-day period to ensure that the MDL determination is more representative of routine measurements as performed in the laboratory.
 - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD (relative standard deviation) values ≤ 20%.
- 4020 B.1.b. – Verify MDL at least **annually**.
 - Ideally use pooled data from several analysts rather than data from one analyst.
- **Real people language – have several operators, who run this test, analyze chlorine or Potassium Permanganate (KMnO₄) standards at a concentration of 0.05 mg/L over several days with a total of at least 7 samples**
 - **Joe analyzes 3 samples on Monday**
 - **Bob analyzes 3 samples on Tuesday**
 - **Mary analyzes 3 samples on Wednesday**
- **Run this once a year**

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves.
- 4020.B.2.a. – Calibrate initially with at least one blank and three calibration standards.
 - The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
 - The back-calculated and true concentrations should agree within ± 10%.

- **Real people language** – prepare a set of chlorine or potassium permanganate (KMnO_4) standards in accordance with [Guidance for Secondary Standards Use in Calibration 12-19-2013](#) monthly.

Method Blank

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure.
- 4020 B.2.d. – Include at least one method blank *daily* or with each batch of 20 or fewer samples, whichever is more frequent.
 - If any method blanks measurements are at or above the reporting level, take immediate corrective action.
- **Real people language** – analyze distilled water as a sample by adding a DPD powder pillow and waiting the 3-6 minutes before reading
 - **Target value is less than reporting limit**
 - **Reporting limit will be equal to your Method Detection Limit (MDL)**
 - **Run on a 5% basis (see batch size for more information).**

Laboratory Fortified Blank (LFB)

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.
 - Sample batch = 5% basis = 1 every 20 samples
 - Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve.
- 4020 B.2.e. – Calculate percent recovery, plot control charts and determine control limits
- **Real people language** – analyze chlorine or potassium permanganate standard at a concentration of 0.5 mg/L
 - **Run on a 5% basis (see batch size for more information).**

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD)

- NONE

Duplicate

- 1020 B.12.f. – Calculate RPD (relative percent difference)
- 4020 B.2.f. – Randomly select routine samples to be analyzed twice.
 - Process duplicate sample independently through the entire sample preparation and analysis.
 - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples.
- **Real people language** – on a 5% basis (see batch size for more information) analyze 2 samples for chlorine, after reading one, pour out sample and start with a fresh sample
 - **For reporting purposes, average sample and duplicate.**
 - **Target value should be close to the first value and have a small RPD (less than 20%)**

Continuing Calibration Verification (CCV)

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
 - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
 - For the calibration verification to be valid, check standards must not exceed 10% of its true value
- **Real people language**
 - **Read Secondary Standards in accordance with [Guidance for Secondary Standards Use in Calibration 12-19-2013](#) daily (day of).**
 - **OR run a chlorine or potassium permanganate standard daily.**

Control Charts – 1020 B.13.

- **Real people language**
 - **Create and maintain control charts if you have 20-30 data points within 90 days.**
 - **If you do not meet the above criteria, follow QC Acceptance Criteria below.**

Corrective Action - 1020 B.5., B.8., & B.15.**QC Acceptance Criteria**

- Blanks < Method Detection Limit (MDL)
- LFB \pm 15%
- ICV/CCV \pm 10%
- RPD < 20%
- Reporting Limit = MDL

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If sampling only once a month, need to run QC once a month (when samples are analyzed).

Calculations

- % Recovery for LFB
 - = $\frac{\text{LFB concentration}}{\text{Expected concentration}} \times 100\%$

Total Residual Chlorine

TDEC – Fleming Training Center

S. Pratt, January 2014



- RPD – relative percent differences for duplicates
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$

The Use of Secondary Standards for Spectrophotometer/Colorimeter Calibration

Secondary standards (gel standards) are specifically designed to verify the instrument's calibration and to check the instrument's performance. They are not intended to be used to create calibration curves or to calibrate the instrument. Because the DPD reagent cannot be mixed with the gel standards, the quality and the reaction time of the reagent cannot be assessed. For these reasons gel standards cannot take the place of primary standards.

The analyst is responsible for the following:

- Preparing the calibration curve for each instrument ***once per month*** at a minimum with chlorine standards or potassium permanganate (see instructions below for KMnO_4), before the use of new DPD reagents, or the use of new gel standards
- Recording reagent lot #'s for reagents and standards
- Recording calibration concentrations
- Verifying the calibration curve using a minimum of one blank and two gel standards that bracket the expected sample concentration
- Recording all verification data

POTASSIUM PERMANGANATE (KMnO_4) STOCK STANDARD SOLUTION

0.891 grams of reagent grade KMnO_4 in 1000 mL vol. flask made to mark with deionized water. Deionized water must never be stored in plastic containers or exposed to airborne contamination. Store the stock solution in an amber bottle in a cool area. The typical shelf life of the stock solution is six (6) months. If solids appear in the solution, **do not use**.

*****Avoid leaving the cap off for extended periods of time and avoid contamination.*****

INTERMEDIATE (WORKING) STANDARD SOLUTION (10 mg/L)

10 mL of *STOCK* made in 1000 mL vol. flask made to mark with deionized water. The flask should be labeled with the name, KMnO_4 , date of preparation, and initials of who made it.

This information should also be entered into a logbook.

****The intermediate stock solution should be stable for approximately 5 days if kept cool and away from light.****

Care should be taken that the pipette and glassware are clean and thoroughly rinsed with deionized water to avoid contamination. Store only in a glass container (preferably amber glass) never in plastic containers. The working solution should be remade if solids appear in the bottom of the container.

CALIBRATION STANDARD SOLUTIONS

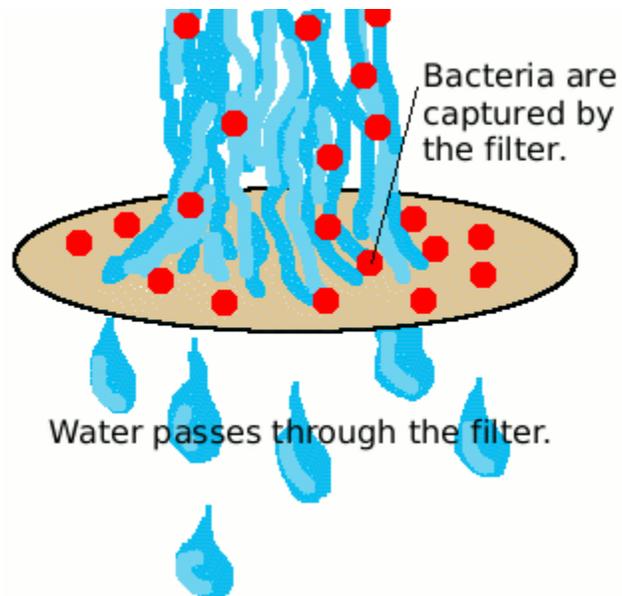
If using KMnO_4 , four to five calibration standard solutions should be made according to the table below with the addition of DPD to create a calibration curve ***once per month*** at a minimum. The correlation coefficient of the curve should correlate to 0.995 or better. This curve is then used to check instrument calibration. Gel standards are run against the curve and must agree to within $\pm 10\%$.

****The working solution should be stable for approximately 2 hours and will fade rapidly (within 15 minutes) if chlorine demand-free water is not used.****

A target value (e.g. permit value for a facility) should be known, and three gel standards, 0.00 mg/L (blank) and two other standards (a low and a high standard) that bracket the target value should be chosen. Gel standards are run against the curve and must agree to within $\pm 10\%$.

mL Working Standard Diluted w/Deionized water	Chlorine Equivalent mg/L
20 mL (vol. Pipette) to 100 mL (vol. flask)	2.0 mg/L
10 mL (vol. Pipette) to 100 mL (vol. flask)	1.0 mg/L
5 mL (vol. Pipette) to 100 mL (vol. flask)	0.5 mg/L
1 mL (vol. Pipette) to 100 mL (vol. flask)	0.1 mg/L
1 mL (vol. Pipette) to 200 mL (vol. flask)	0.05 mg/L
1 mL (vol. Pipette) to 500 mL (vol. flask)	0.02 mg/L
100 mL of deionized water	0.00 mg/L

Section 2 Bacteriological





BACTERIOLOGICAL ANALYSIS
Intermediate Wastewater Lab

1

TDEC - Fleming Training Center

COLIFORM BACTERIA

- MPN of coliform bacteria are estimated to indicate the presence of bacteria originating from the intestines of warm-blooded animals
- Coliform bacteria are generally considered harmless
 - But their presence may indicate the presence of pathogenic organisms

2

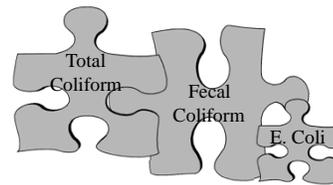
COLIFORM BACTERIA

- Comprised of all the aerobic and facultative anaerobic gram negative, nonspore-forming, rod-shaped bacteria that ferment lactose within 48 hours at ~35°C
- Coliform bacteria can be split into fecal and non-fecal groups
 - The fecal group can grow at higher temperatures (~45°C) than the non-fecal coliforms

3

FAMILY PORTRAIT

- Indicators of water contamination



4

SAMPLING

- Clean, sterilized borosilicate glass or plastic bottles or sterile plastic bags.
- Leave ample air space for mixing.
- Collect samples representative of wastewater tested.
- Use aseptic techniques; avoid sample contamination.
- Test samples as soon as possible.

5

SAFETY

- Follow standard safety practices appropriate to microbiological laboratories.
- Materials suspected of containing viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding.



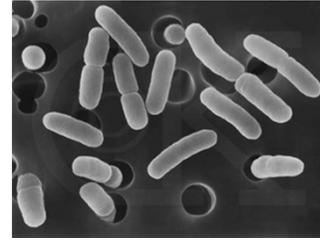
6

APPROVED METHODS

- Coliform (fecal)
 - Number per 100 mL
 - Membrane filtration
- E. coli
 - Number per 100 mL
 - Multiple tub/multiple well (Colilert®)
 - Membrane filtration
 - m-ColiBlue24®
 - Modified mTEC agar

TDEC - Fleming Training Center

7



FECAL COLIFORM

8

TDEC - Fleming Training Center

SUMMARY OF METHOD

- A 100 mL volume of sample is filtered through a 47-mm membrane filter using standard techniques.
- Filter is transferred to a 50-mm petri plate containing an absorbent pad saturated with mFC Broth.
- Invert filter and incubate at $44.5 \pm 0.2^\circ\text{C}$ for 24 hrs.
- Count **blue** colonies.

TDEC - Fleming Training Center

9

INTERFERENCES

- No interferences
- Excess particulates may cause colonies to grow together on a crowded filter or slow the sample filtration process.

TDEC - Fleming Training Center

10

EQUIPMENT



- Water bath or air incubator operating at $44.5 \pm 0.2^\circ\text{C}$
- Vacuum pump
- UV sterilizer or boiling water bath
- 10-15 X dissecting microscope; should have fluorescent illuminator
- Alcohol burner

TDEC - Fleming Training Center

11

SUPPLIES AND GLASSWARE

- Sterile graduated cylinder
- Sterile pipets
- Sterile MF filtration flask
- Sterile dilution water
- Sterile sample vessels
- Samples containing chlorine must be treated with 3% sodium thiosulfate solution
- mFC Broth



TDEC - Fleming Training Center

12

SAMPLING

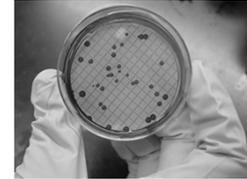
- Maximum hold time is 8 hrs at $< 10^{\circ}\text{C}$
- Ideal sample volume yields 20-60 colonies
- Samples < 20 mL, add 10 mL sterile dilution water to filter funnel before applying vacuum.
- Sterilize funnel between samples.

TDEC - Fleming Training Center

13

FECAL DATA ANALYSIS

- Visually determine colony counts on membrane filters.
- Verify using 10-15 X binocular wide-field microscope.
- Fecal coliforms appear blue.



TDEC - Fleming Training Center

14

FECAL DATA INTERPRETATION

- Incubation time is 24 ± 2 hrs.
- Fecal coliform density reported as number of colonies per 100 mL of sample.
- NPDES permit limit: monthly average of 200/100 mL; daily maximum of 1000/100 mL.

TDEC - Fleming Training Center

15

APPENDIX C: PATHOGEN REDUCTION ALTERNATIVES FOR CLASS B BIOSOLIDS

- Class B—Alternative 1
 - (i) Seven representative samples of the biosolids that are applied to the land shall be collected.
 - (ii) The geometric mean of the density of fecal coliform in the samples collected in subpart (i) of this part shall be less than either 2,000,000 Most Probable Number per gram of total solids (dry weight basis) or 2,000,000 Colony Forming Units per gram of total solids (dry weight basis).

TDEC - Fleming Training Center

16



ESCHERICHIA COLI (E.COLI)
Colilert

17

TDEC - Fleming Training Center

COLILERT® & COLILERT-18®

MPN Method



- Add substrate to a 100 mL sample
- If making dilutions, use sterile DI water, not sterile buffered water.

TDEC - Fleming Training Center

18



ESCHERICHIA COLI (E.COLI)
Modified mTEC Agar with Membrane Filtration

25

TDEC - Fleming Training Center

EPA METHOD 1603

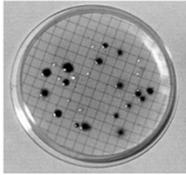
- Membrane Filter – modified mTEC agar
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)
- Place sample in a petri dish with modified mTEC agar
- Invert dish and incubate for **35± 0.5°C for 2 hours**
 - Resuscitates injured or stressed bacteria
- Then incubate at **44.5± 0.2°C for 22 hours**
- After incubation, remove the plate from the water bath or dry air incubator
- Daily QC adds quite a bit to this test

26

METHOD 1603

- Count and record the number of **red or magenta** colonies (verify with stereoscopic microscope)

Modified mTEC



Count magenta colonies as *E. coli*. These are easily discerned from non-target colonies which are clear or beige.

- See the USEPA microbiology methods manual, Part II, Section C, 3.5, for general counting rules

27

TDEC - Fleming Training Center

METHOD 1603

- QC Tests:
 - Initial precision and recovery
 - Ongoing precision and recovery
 - Matrix spike
 - Negative control
 - Positive control
 - Filter sterility check
 - Method blank
 - Filtration blank
 - Media sterility check

28

METHOD 1603

- Initial precision and recovery
 - Should be performed by each lab before the method is used for monitoring field samples
- Ongoing precision and recovery
 - Run after every 20 field and matrix spike samples or one per week that samples are analyzed
- Matrix spike
 - Run 1 per 20 samples

29

TDEC - Fleming Training Center

METHOD 1603

- Negative control
 - Should be analyzed whenever a new batch of media or reagents is used
- Positive control
 - Should be analyzed whenever a new batch of media or reagents is used
- Filter sterility check
 - Place at least one membrane filter per lot of filters on a tryptic soy agar (TSA) plate and incubate for 24 ± 2 hours at 35°C ± 0.5°C .
 - Absence of growth indicates sterility of the filter.
 - Run **daily**.

30

METHOD 1603

- Method blank
 - Filter a 50-mL volume of sterile buffered dilution water and place on a modified mTEC agar plate and incubate.
 - Absence of growth indicates freedom of contamination from the target organism.
 - Run ***daily***.
- Filtration blank
 - Filter a 50-mL volume of sterile buffered dilution water and place on a TSA plate and incubate at just at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours .
 - Absence of growth indicates sterility of the buffer and filtration assembly.
 - Run ***daily***.

TDEC - Fleming Training Center

31

METHOD 1603

- Media sterility check
 - The lab should test media sterility by incubating one unit (tube or plate) from each batch of medium (TSA, modified mTEC and verification media) as appropriate and observing for growth.
 - Absence of growth indicates media sterility.
 - Run ***daily***.

TDEC - Fleming Training Center

32



ESCHERICHIA COLI (E.COLI)
m-ColiBlue24® with Membrane Filtration

33

TDEC - Fleming Training Center

M-COLIBLUE24®

- Membrane Filter
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)



TDEC - Fleming Training Center

M-COLIBLUE24®

- Place sample in a petri dish with absorbent pad containing 2 mL mColiBlue 24 broth
- Invert dish and incubate at $35 \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours

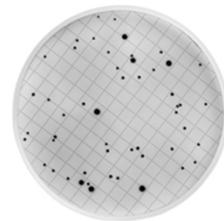


TDEC - Fleming Training Center

35

M-COLIBLUE24®

- After incubation, remove the plate from the water bath or dry air incubator
- Count and record the number of **blue** colonies (verify with stereoscopic microscope)
- See the USEPA microbiology methods manual, Part II, Section C, 3.5, for general counting rules



TDEC - Fleming Training Center

36

E. COLI DATA ANALYSIS

- Maximum sample hold time: 6 hrs
- Samples and equipment known or suspected to have viable E. coli attached or contained must be sterilized prior to disposal.

TDEC - Fleming Training Center

37

E. COLI DATA INTERPRETATION

- Permit limit: 126 colonies/100 mL monthly average; or daily max of 487 or 941/100 mL depending on permit
- For MF method:
 - Select sample volumes to produce 20-80 colonies on the membranes.
 - Run minimum of 3 dilutions.
 - Must use sterile buffered water for dilutions and to rinse filtration unit.

TDEC - Fleming Training Center

38

EXPECTED REACTIONS OF VARIOUS MICROORGANISMS

- Total coliforms will produce a red colony
 - Enterobacter species
 - *E. cloacae*
 - *E. aerogenes*
 - Klebsiella species
 - *K. pneumoniae*
 - Citrobacter species
 - *C. freundii*
- *Escherichia coli* will produce a blue colony
 - *E. coli* O157:H7 will not produce a blue colony, but will grow as a red colony

TDEC - Fleming Training Center

39

EXPECTED REACTIONS OF VARIOUS MICROORGANISMS

- Known negative reaction (no growth) after 24-25 hours
 - *Pseudomonas aeruginosa*
 - Variable reaction may be positive for total coliform when incubated longer than 25 hours
 - *Proteus vulgaris*
 - *Aeromonas hydrophila*

TDEC - Fleming Training Center

40

EXPECTED REACTIONS OF VARIOUS MICROORGANISMS

- Some strains of the following microorganisms are known to produce a false-positive total coliform reaction (a red colony, but not a true total coliform)

• <i>Serratia species</i>	• <i>Yersinia enterocolitica</i>
• <i>Hafnia alvei</i>	• <i>Leclercia adecarboxylata</i>
• <i>Vibrio fluvialis</i>	• <i>Ewingella americana</i>
• <i>Aeromonas species</i>	• <i>Staphylococcus species</i>
• <i>Proteus vulgaris</i>	• <i>Proteus mirabilis</i>
• <i>Providencia stuartii</i>	

TDEC - Fleming Training Center

41

M-ColiBlue24® Trouble-Shooting Guide, Hach Company, www.Hach.com

E. COLI INFORMATION

- For Colilert®: IDEXX Laboratories, www.idexx.com
- For mTEC Agar and mColiBlue-24® media: Hach Company, www.Hach.com
- EPA Method 1603: E.coli In Water By Membrane Filtration Using Modified-Thermotolerant Escherichia coli Agar (Modified mTEC), September 2002, EPA-821-R-02-023

TDEC - Fleming Training Center

42

E. COLI

- Two Approved Methods
 - SM 9223 B – 2004 IDEXX Colilert Quanti-Tray
 - Hach Method 10029 – m -ColiBlue24® -



TDEC - Fleming Training Center

43

SM 9020 B. QC GUIDELINES

- General Considerations
 - The program must be practical and require only a reasonable amount of time or it will be bypassed.
- Facilities
 - Provide a dust and draft free lab that has a stable temperature that does not have extreme temperature variations.
 - Minimize through traffic and visitors
 - Provide adequate space for conducting the analysis
 - Keeps work area clean and disinfected

TDEC - Fleming Training Center

44

SM 9020 B. QC GUIDELINES

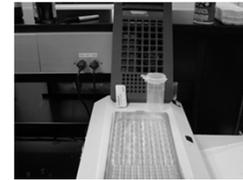
- Lab Equipment
 - Verify thermometer accuracy annually. (~LabtronX or other certification vendor) 9020 B.4.a
 - UV lamps – 9020 B.4.1 (if used)
 - Clean monthly with soft cloth moistened with ethanol
 - Recommend replacing bulbs annually
 - Incubators – 9020 B.4.o
 - Verify thermometers ~ annually
 - Record temperature twice daily (day of), at least 4 hours apart
 - Verify that cold samples incubate for specified time. May need to warm samples in very cold weather
 - Protect incubator from extreme room temperatures. Ideal is 60-80°F

TDEC - Fleming Training Center

45

SM 9020 B. QC GUIDELINES

- Lab Equipment - continued
 - Media
 - Check reagent media appearance with each use and discard if there is a color change.
 - Protect reagent media from light
 - Refrigerators – 9020 B.4.i
 - Maintain temperature at 2-8°C
 - Check and record temps daily (day of)



TDEC - Fleming Training Center

46

SM 9020 B. QC GUIDELINES

- Lab Equipment – continued
 - Membrane Filtration Equipment (if MF procedure is used) – 9020 B.4.k
 - Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize
 - UV sterilize or boil funnel apparatus between samples
 - If using boiling water, make sure membrane filtration equipment is cool before adding next sample

TDEC - Fleming Training Center

47

SM 9020 B. QC GUIDELINES

- Lab Equipment – continued
 - Autoclave – 9020 B.4.h
 - For routine use, verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached
 - Test **monthly** for sterilization efficacy (with *Geobacillus stearothermophilus*)
 - If any media, bottles, filters or other equipment that comes into contact with the samples are sterilized in the autoclave, the sterilization efficacy test must be performed **monthly**
 - If you are only using your autoclave to sterilize waste, you just need an MRT (maximum registering thermometer)

TDEC - Fleming Training Center

48

SM 9020 B. QC GUIDELINES

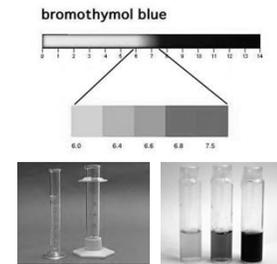
- Lab Equipment – continued
 - Membrane filters and pads (if MF procedure is used) – 9020 B.5.i.3
 - Check filters for brittleness if lot is held for one or more years

TDEC - Fleming Training Center

49

SM 9020 B. QC GUIDELINES

- Lab Supplies
 - Glassware – 9020 8.5.a
 - pH check to test clean glassware for alkaline or acid residue, add a few drops of 0.04% bromothymol blue (BTB) or other pH indicator and observe the color reaction.
 - BTB should be blue-green in the acceptable neutral range



TDEC - Fleming Training Center

50

SM 9020 B. QC GUIDELINES



- Lab Supplies – continued
 - Dilution water bottles – 9020 B.5.c
 - Dilution waters available commercially are acceptable.
 - Check one per lot for pH and volume (99 ± 2 mL) and examine bottles for a precipitate
 - Discard by expiration date
 - Before use of each batch conduct sterility (one bottle per batch) – More information on slide #55

TDEC - Fleming Training Center

51

SM 9020 B. QC GUIDELINES

- Lab Supplies – continued
 - Dilution water bottles sterility check - continued
 - Sterility Checks – 9020B.9.d
 - Check each new batch (or lot) of buffered water for sterility before first use by adding 50 mL of water to 50 mL of a double-strength broth (e.g. tryptic soy, trypticase soy or tryptose broth).
 - Alternatively, aseptically pass 100 mL of dilution water through a membrane filter and place filter on nonselective medium.
 - Incubate at $35 \pm 0.5^\circ\text{C}$ for 24 hours and observe for growth.
 - For membrane filter tests, check the sterility of the entire process by using sterile reagent or dilution water as the sample at the beginning and end of each filtration series of samples and test for growth

TDEC - Fleming Training Center

52

SM 9020 B. QC GUIDELINES

- Lab Supplies – continued
 - Sample bottles – 9020 B.5.d
 - Minimally test for sterility one sample bottle per batch sterilized in the lab. Document results. – More information on slide #55
 - Check accuracy of 100 mL mark, one per lot and record results.

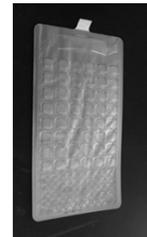


TDEC - Fleming Training Center

53

SM 9020 B. QC GUIDELINES

- Lab Supplies – continued
 - Multi-well trays and sealers – 9020 B.5.e
 - 2014 Update– analyze a method blank once per lot (of sterile water, media, bottles and trays) or once per quarter, whichever is more frequent, to demonstrate sterility.



TDEC - Fleming Training Center

54

SM 9020 B. QC GUIDELINES

- Lab Supplies – continued
 - Multi-well trays and sealers – 9020 B.5.e
 - Evaluate sealing performance of heat sealer unit **monthly** by adding one to two drops of food-color dye to 100 mL deionized water sample, run through sealer, and visually check each well for leakage.
 - 2014 Update** – As a monthly check of a sealer efficacy, perform and document a visual check that trays are properly sealed. If all sample wells are positive for total coliform and sufficient contrast, visually examine the tray cells for leakage and document the check. If insufficient color contrast is present use food-color dye as previously recommended by method.
 - Perform cleaning and maintenance on sealer annually or more frequently, if needed.

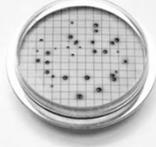


TDEC - Fleming Training Center

55

SM 9020 B. QC GUIDELINES

- Coliforms – Total and E. coli Hach Metho – m-ColiBlue24®
 - Blank – daily (day of)
 - Run at least one membrane filter blank at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter, placing in a petri dish with mColiBlue broth and testing for growth.
 - Positive and Negative Controls – Check certified control cultures with each lot of media and petri dishes with pads OR once a quarter, whichever is more frequent.
 - Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control.
 - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.



TDEC - Fleming Training Center

56

SM 9020 B. QC GUIDELINES

- Enzyme Substrate Test SM 9223 B, 22nd Edition (2004) – Colilert Method
 - Quality Control
 - Test **each lot of media or quarterly (whichever is more frequent)** purchased for performance by inoculation with two certified control bacteria: *Escherichia coli* and a noncoliform.
 - Also add a sterile water control. If a sterile water control exhibits faint fluorescence or faint positive coliform, discard use and use a new batch of substrate.
 - Incubate these controls at 35±0.5°C as indicated above.
 - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.

TDEC - Fleming Training Center

57

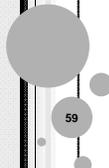
SM 9020 B. QC GUIDELINES

- Reporting Duplicates
 - Both results should be documented on bench sheet.
 - All duplicates should be reported according to your permit limits. If your permit sets a maximum limit, then the maximum value should be reported even if falls outside your permit limit.
 - If both values fall below your daily max, average (arithmetically) the daily duplicates to get one result and then using that averaged result as part of the monthly geometric mean calculation.

TDEC - Fleming Training Center

58

MEMBRANE FILTER COUNTS



TDEC - Fleming Training Center

59

WITHIN ACCEPTABLE LIMITS

$$\text{Count per 100 mL} = \frac{\text{Number of colonies}}{\text{Vol. of sample filtered (mL)}} \times 100$$

- Assume that filtration of volumes 50, 15, 5, 1.5 and 0.5 mL produced colony counts of 200, 110, 40, 10 and 5 respectively
- You do not need to count the colonies on all the filters. Select the membrane filters (MF's) with 20-60 Fecal Coliforms and 20-80 E. coli colonies

TDEC - Fleming Training Center

60

WITHIN ACCEPTABLE LIMITS

$$\text{Count per 100 mL} = \frac{\text{Number of colonies}}{\text{Vol. of sample filtered (mL)}} \times 100$$

- After selecting the best MF's with a 40 colony count, you apply the general formula as follows

$$\text{Count per 100 mL} = \frac{40}{5} \times 100 = 800/100 \text{ mL}$$

TDEC - Fleming Training Center

61

MORE THAN ONE ACCEPTABLE COUNT

- If there are acceptable counts on replicate plates, carry counts independently to final reporting units, then calculate the arithmetic mean of these counts to obtain the final reported value

TDEC - Fleming Training Center

62

MORE THAN ONE ACCEPTABLE COUNT

- For example, 1 mL volumes produce coliform counts of 26 and 36 or counts of 2600 and 3600/100 mL

$$\frac{2600 + 3600}{2} = 3100/100 \text{ mL}$$

TDEC - Fleming Training Center

63

MORE THAN ONE ACCEPTABLE COUNT

- If more than one dilution, independently carry counts to final reporting units, then average for final reported value

TDEC - Fleming Training Center

64

MORE THAN ONE ACCEPTABLE COUNT

- For example, assume that volumes of 0.3, 0.05, 0.03 and 0.01 mL produced coliform colony counts of TNTC (Too Numerous To Count), 55, 30 and 8 respectively.
- In this example, two volumes, 0.05 and 0.03 produce colonies in the acceptable counting range

TDEC - Fleming Training Center

65

MORE THAN ONE ACCEPTABLE COUNT

- Independently carry each MF count to a count per 100 mL

$$\frac{55}{0.05} \times 100 = 110,000/100 \text{ mL} \quad \frac{30}{0.03} \times 100 = 100,000/100 \text{ mL}$$

- Then calculate the arithmetic mean of these counts to obtain the final reported value

$$\frac{110,000 + 100,000}{2} = 105,000/100 \text{ mL}$$

TDEC - Fleming Training Center

66

IF ALL MF COUNTS ARE BELOW THE LOWER LIMIT

- Select the most nearly acceptable count
- For example, assume a count in which sample volumes of 1, 0.3 and 0.01 mL produced colony counts of 14, 3 and 0 respectively
- Here, no colony count falls within the recommended limits.
 - Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark

$$\frac{14}{1.0} \times 100 = 1400/100 \text{ mL}$$

67

TDEC - Fleming Training Center

IF ALL MF COUNTS ARE BELOW THE LOWER LIMIT

- Here, no colony count falls within the recommended limits.
 - Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark

$$\frac{14}{1.0} \times 100 = 1400 \quad \text{Report as: estimated 1400/100mL}$$

68

TDEC - Fleming Training Center

IF COUNTS FROM ALL MF ARE ZERO

- Calculate using count from largest filtration volume
- For example, sample volumes of 25, 10 and 2 mL produced colony counts of 0, 0 and 0 respectively and no actual calculation is possible, even as an estimated report.

69

TDEC - Fleming Training Center

IF COUNTS FROM ALL MF ARE ZERO

- Calculate the number of colonies per 100 mL that would have been reported if there had been one colony on the filter representing the largest filtration volume

$$\frac{1}{25} \times 100 = 4 \quad \text{Report as: < (Less than) 4/100mL}$$

70

TDEC - Fleming Training Center

IF ALL MEMBRANE COUNTS ARE ABOVE THE UPPER LIMIT

- Calculate the count with the smallest volume filtered
- For example, assume that the volumes 1, 0.3 and 0.01 mL produced colony counts of TNTC, 150 and 110.

71

TDEC - Fleming Training Center

IF ALL MEMBRANE COUNTS ARE ABOVE THE UPPER LIMIT

- Since all colony counts are above the recommended limit, use the colony count from the smallest sample volume filtered and estimate the count as

$$\frac{110}{0.01} \times 100 = 1,100,000 \quad \text{Report as: estimated 1,100,000/100 mL}$$

72

TDEC - Fleming Training Center

IF COLONIES ARE TOO NUMEROUS TO COUNT

- Use upper limit with smallest filtration volume
- For example, assume that the volumes 1.0, 0.3 and 0.01 mL all produced too many colonies to show separated colonies and that the laboratory bench records showed TNTC

TDEC - Fleming Training Center

73

IF COLONIES ARE TOO NUMEROUS TO COUNT

- Use 60 colonies for Fecals and 80 for E. coli as the basis of calculation with the smallest filtration volume

$$\frac{60}{0.01} \times 100 = 600,000 \quad \text{Report as: } > \text{ (Greater Than)} \\ \text{600,000/100 mL}$$

TDEC - Fleming Training Center

74

CALCULATING GEOMETRIC MEAN

- When there are individual sample results that are reported as <, > or estimated
 - If any individual sample result is reported as an estimate, drop the estimate when calculating the geometric mean

TDEC - Fleming Training Center

75

CALCULATING GEOMETRIC MEAN

- When there are individual sample results that are reported as <, > or estimated
 - If there are any individual samples reported as <, drop the < signs when calculating the geometric mean
 - However, report the geometric mean as a < value

TDEC - Fleming Training Center

76

CALCULATING GEOMETRIC MEAN

- When there are individual sample results that are reported as <, > or estimated
 - If there are any individual samples reported as >, drop the > signs when calculating the geometric mean
 - However, report the geometric mean as a > value

TDEC - Fleming Training Center

77

CALCULATING GEOMETRIC MEAN

- When there are individual sample results that are reported as <, > or estimated
 - If there are samples reported as < and one or more samples reported as >, drop the < and > signs when calculating the geometric mean
 - However, report the geometric mean as a > value

TDEC - Fleming Training Center

78

EPA Microbiological Methods for Monitoring the Environment Water and Wastes, EPA-600/8-78-017, December 1978

Bacteria, Coliform

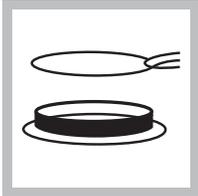
Using m-ColiBlue24 Broth PourRite Ampules

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages; surface, well, and groundwater, waste water, recreational waters, and process water for ultrapure, chemical processing and pharmaceutical applications.



Simultaneous Total Coliform and E.coli Screening

Method 10029



1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish.

Note: Do not touch the pad or the inside of the petri dish.

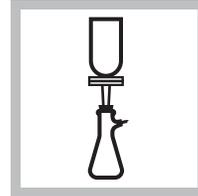
Note: To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.



2. Invert ampules two or three times to mix broth. Break open an ampule of m-ColiBlue24 Broth using an ampule breaker. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

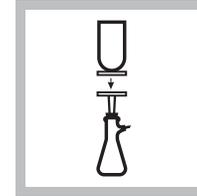


3. Set up the Membrane Filter Apparatus. With sterile forceps, place a membrane filter, grid side up, into the assembly.

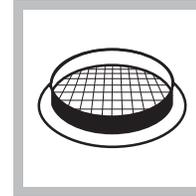


4. Shake the sample vigorously to mix. Pour 100 mL of sample or diluted sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls three times with 20 to 30 mL of sterile buffered dilution water.

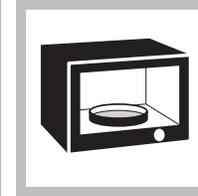
Bacteria, Coliform



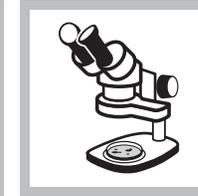
5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.



7. Invert the petri dish and incubate at $35 \pm 0.5^\circ\text{C}$ for 24 hours.



8. Remove the petri dish from the incubator and examine the filters for colony growth. Colonies are typically readily visible; however, a stereoscopic microscope or other 10–15X magnifier may be useful. Red and blue colonies indicate total coliforms and blue colonies specifically indicate *E. coli*.

Note: Sometimes only the center of a colony will be colored. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as a blue colony. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as *E. coli*.

Optional Testing of Red Colonies

The m-ColiBlue24 Broth is formulated so that coliforms other than *E. coli* grow as red colonies. The percentage of red colonies that are false positives (non-coliforms) is comparable to the percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms); therefore, confirmation is not required.

A few varieties of the non-coliform bacteria *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. may grow on m-ColiBlue24 Broth and form red colonies. Such bacteria can be readily distinguished from total coliforms by the oxidase test. *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If your sample contains high levels of interfering bacteria, you can perform an oxidase test to confirm which red colonies are total coliforms.

Quality Assurance for E. coli Analysis

Laboratory Equipment and Instrumentation

- Thermometers – 9020B.4.a
 - Annually check accuracy of all working temperature-sensing devices... against a certified NIST thermometer or one traceable to NIST and conforming to NIST specifications.
 - Record calibration results, along with the date and the technician's signature, in a quality control logbook.
 - Mark the necessary calibration correction factor on each temperature measuring device so that only calibrated-corrected temperature values are recorded.
 - Verify accuracy of the reference certified thermometer as specified on the certificate of calibration or at least every 5 years.
 - For general purposes use thermometers graduated in increments of 0.5°C or less.
- Autoclave – 9020B.4.h
 - For routine use, verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached.
 - Test monthly for sterilization efficacy (with *Geobacillus stearothermophilus*)
 - Verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached.
- Refrigerator – 9020B.4.i
 - Maintain temperature at 2-8°C
 - Check and record temperature daily
- Membrane filtration equipment (if MF procedure is used) – 9020B.4.k
 - Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize.
 - UV sterilize or boil funnels between samples
 - If using boiling water, make sure membrane filtration equipment is cool before adding next sample
- Membrane filters and pads (if MF procedure is used) – 9020B.5.i.3
 - Check filters for brittleness if lot is held for one or more years
- Ultraviolet lamps (if used) – 9020B.4.l
 - When used, disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol
- Incubator – 9020 B.4.o
 - During usage periods check and record calibration-corrected temperature twice daily (morning and afternoon, separated by at least 4 hours) on each shelf in use to ensure temperature consistency throughout unit.

Laboratory Supplies

- Glassware – 9020 B.5.a
 - 1) pH check - To test clean glassware for alkaline or acid residue add a few drops of 0.04% bromthymol blue (BTB) or other pH indicator and observe the color reaction.
 - BTB should be blue-green (in the acceptable neutral range).
- Dilution water bottles – 9020 B.5.c
 - Dilution waters available commercially are acceptable.

E. coli

TDEC – Fleming Training Center

S. Pratt, January 2014



- Check one per lot for pH and volume (99 ± 2 mL) and examine bottles for a precipitate
- Discard by expiration date
- Before use of each batch or lot conduct sterility (one bottle per lot or quarter with that same lot number, whichever is more frequent)
 - Sterility Checks – 9020B.9.d
 - Check each new batch (or lot) of buffered water for sterility before first use by adding 50 mL of water to 50 mL of a double-strength broth (e.g. tryptic soy, trypticase soy or tryptose broth).
 - Alternatively, aseptically pass 100 mL of dilution water through a membrane filter and place filter on nonselective medium.
 - Incubate at $35 \pm 0.5^\circ\text{C}$ for 24 hours and observe for growth.
 - For membrane filter tests, check the sterility of the entire process by using sterile reagent or dilution water as the sample at the beginning and end of each filtration series of samples and test for growth
- Sample bottles – 9020 B.5.d.
 - Check accuracy of 100 mL mark, one per lot and record results.
- Multi-well trays and sealers – 9020 B.5.e
 - Evaluate sealing performance of heat sealer unit monthly by adding one to two drops of food-color dye to 100 mL deionized water sample, run through sealer and visually check each well for leakage.
 - **Real people language – analyze a method blank once per lot (of sterile water, media, bottles and trays) or once per quarter, whichever is more frequent, to demonstrate sterility.**
 - **As a monthly check of a sealer efficacy, perform and document a visual check that trays are properly sealed. If all sample wells are positive for total coliform and sufficient contrast, visually examine the tray cells for leakage and document the check. If insufficient color contrast is present use food-color dye as previously recommended by method.**

General QC Requirements

- Coliforms – Total and E. coli Hach Method 10029 – m-ColiBlue24®
 - Blank – daily
 - Run at least one membrane filter blank at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter, placing in a petri dish with mColiBlue broth and testing for growth.
 - Positive and Negative Controls – Check certified control cultures with each lot of media **and** petri dishes with pads OR once a quarter, whichever is more frequent.
 - *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control.
 - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.
- Enzyme Substrate Test SM 9223 B, 22nd Edition (2004) – Colilert Method
 - Quality Control

E. coli

TDEC – Fleming Training Center

S. Pratt, January 2014

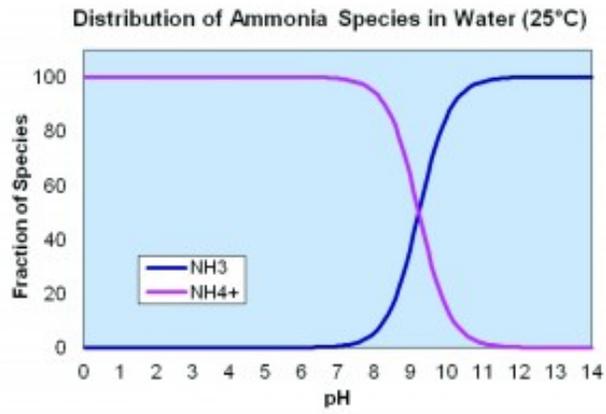


- Test each lot of media or quarterly (whichever is more frequent) purchased for performance by inoculation with two certified control bacteria: *Escherichia coli* and a noncoliform.
- Also add a sterile water control. If a sterile water control exhibits faint fluorescence or faint positive coliform, discard use and use a new batch of substrate.
- Incubate these controls at $35\pm 0.5^{\circ}\text{C}$ as indicated above.
- Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.

Bibliography

American Public Health Association (APHA), American Waterworks Association (AWWA), and Water Environment Federation (WEF). 2012. *Standard Methods for the Examination of Water and Wastewater*. 22nd ed. American Public Health Association, Washington, D.C.

Section 3 Ammonia



Ammonia Nitrogen

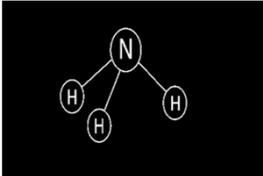
Wastewater Lab



TDEC - Fleming Training Center 1

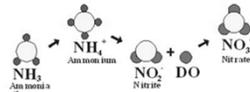
Ammonia Nitrogen

- In domestic wastewater is generally between 10-40 mg/L.
- Primary treatment may increase the ammonia nitrogen slightly due to the composition of some protein compounds during treatment



TDEC - Fleming Training Center 2

Ammonia Nitrogen



NH₃ Ammonia (from urea, manure and feces)
 NH₄⁺ Ammonium
 NO₂⁻ Nitrite
 NO₃⁻ Nitrate
 DO Dissolved Oxygen

- During secondary treatment processes, ammonia may be oxidized to nitrite then to nitrate in varying degrees depending on factors such as wastewater temperature, residence time of the microorganisms and oxygen amounts.

TDEC - Fleming Training Center 3

Nitrification – The reaction

- $2\text{NH}_3 + \text{HCO}_3^- + 3\text{O}_2 \xrightarrow{\text{Nitrosomonas}} 2\text{HNO}_2 + 4\text{H}_2\text{O} + 2\text{CO}_2$

+

- $2\text{HNO}_2 + \text{O}_2 + 2\text{HCO}_3^- \xrightarrow{\text{Nitrobacter}} 2\text{NO}_3^- + 2\text{H}_2\text{O} + 2\text{CO}_2$

The Nitrobacter microorganisms use the nitrite in nitrous acid oxidizing it to nitrate.

TDEC - Fleming Training Center 4

Ammonia Nitrogen

- Ammonia levels can
 - Increase chlorine demand
 - Cause fish toxicity
 - Increase oxygen demand on receiving water

TDEC - Fleming Training Center 5

Forms of Nitrogen in Activated Sludge

<ul style="list-style-type: none"> □ Un-Oxidized Forms of Nitrogen <ul style="list-style-type: none"> ■ Nitrogen Gas (N₂) ■ Ammonia ■ Organic Nitrogen including proteins, amino acids, urea, etc.. 	→	<ul style="list-style-type: none"> □ Oxidized Forms of Nitrogen <ul style="list-style-type: none"> ■ Nitrite ■ Nitrate ■ Nitrous Oxide
---	---	---

TDEC - Fleming Training Center 6

More on Nitrogen in Activated Sludge

- Total Nitrogen = TKN + NO₂ + NO₃
- TKN (Total Kjeldahl Nitrogen) = NH₃ + Organic Nitrogen
- Rule of thumb:
 - Ammonia makes up approximately 60% of TKN
 - Organic Nitrogen is typically removed in settled sludge
 - TKN makes up approximately 15% - 20% of the total influent BOD.

TDEC - Fleming Training Center

7

Why Nitrify?

- Ammonia can be harmful if discharged.
 - Creates dissolved oxygen sag in receiving stream
 - Toxic to fish and other aquatic life
 - Possible problem for downstream water supplies.
 - Nutrient input (when oxidized).



Toxic to Fish and Aquatic Life

TDEC - Fleming Training Center

8

Some Ammonia Effects

Ammonia Levels & Effects	
NH ₃ Levels	Effects
0.06 mg/L	Fish can suffer gill damage
0.1 mg/L	Usually indicative of polluted waters
0.2 mg/L	Sensitive fish like trout and salmon begin to die
2.0 mg/L	Ammonia-tolerant fish, like carp, begin to die

The danger ammonia poses for fish depends on the water's temperature and pH. The higher the pH and temperature, the more toxic the ammonia.

TDEC - Fleming Training Center

9

Sources of Ammonia In Wastewater

- Incoming Raw Wastewater (domestic waste)
- Internal Recycle (anaerobic digester supernatant, belt press filtrate) - high
- Septage (high)
- Industrial Sources



TDEC - Fleming Training Center

10

How Much Ammonia ???

- Typically, expect influent domestic wastewater to have 25 – 30 mg/l of NH₃-N
- Considered Strong if > 50mg/l NH₃-N
- Septage 150 mg/l



TDEC - Fleming Training Center

11

Sample Collection

- Collect samples in glass or plastic containers
- Fill sample bottle complete
- If chlorine is present, treat with sodium thiosulfate
 - Add one drop of 0.1N sodium thiosulfate solution for every 0.3 mg/L of chlorine present

TDEC - Fleming Training Center

12

Sample Collection

- Analyze as soon as possible
 - Refrigerate at 4°C for samples to be analyzed within 24 hours
 - If this is not possible, preserve the sample with sulfuric acid to pH < 2 and store at 4°C.
 - Samples acidified and cooled may be stored for 28 days
- Before analysis, neutralize the sample to pH 7 with 5N sodium hydroxide

TDEC - Fleming Training Center

13

Procedural Concerns

- Ammonia distillation apparatus should be steamed out
- A high & low standard should be carried through the ammonia distillation



TDEC - Fleming Training Center

14

Procedural Concerns

- Distillate is caught in boric acid solution for titration or nesslerization
- Distillate is caught in 0.04N H₂SO₄ if using the probe method

TDEC - Fleming Training Center

15

Probe Method

- The ammonia electrode measures ammonia gas or ammonium ions in aqueous solutions that have been converted to gas by the addition of a strong base.
- The electrode is a complete electrochemical cell consisting of a glass pH electrode and a reference electrode.
- The gas-permeable membrane separates the sample from a thin layer of electrolyte that is pressed between the pH bulb and the membrane.

TDEC - Fleming Training Center

16

Probe Method

- At high pH, ammonium ion is converted to ammonia gas.
- The gas diffuses through the membrane and causes a pH change in the thin layer of electrolyte.
- The potential across the pH glass changes as a result of the pH change and the electrode measures the change in potential.
- The measured pH change is proportional to the ammonia concentration in the solution.

TDEC - Fleming Training Center

17

Ammonia SM4500-NH3 D -1997

- 136 Table 1B
 - Distillation Required
 - Footnote #6
 - Comparability Study
 - Follow Standard Methods
- See page 29,784 of the 136 Rule, Footnote #6



TDEC - Fleming Training Center

18

40 CFR 136 05-21-2012 Table 1B

- 21st & 22nd Ed. Method 4500-NH3 D: "Sample distillation is unnecessary."
- Footnote 6 – "Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies. In general, the analytical method should be consulted regarding the need for distillation. If the method is not clear, the laboratory may compare a minimum of 9 different sample matrices to evaluate the need for distillation. For each matrix, a matrix spike and matrix spike duplicate are analyzed both with and without the distillation step. (A total of 36 samples, assuming 9 matrices). If results are comparable, the laboratory may dispense with the distillation step for future analysis. Comparable is defined as < 20% RPD for all tested matrices). Alternatively the two populations of spike recovery percentages may be compared using a recognized statistical test."

TDEC - Fleming Training Center

19

Ammonia SM4500-NH3 D -1997

- Standard Methods
 - 4500-NH3 A.1 – In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater and good-quality nitrified wastewater effluent.
 - 4500-NH3 D.1.b. – Sample distillation is unnecessary.
- Tennessee recommends that one sample is run yearly to compare the distilled and undistilled results and that the results are within 20% of each other.
 - Note – if distilled sample and undistilled sample are below detection limit, you cannot calculate the percent difference.

TDEC - Fleming Training Center

20

Ammonia SM4500-NH3 D -1997

- DOC
- MDL
- LRB
- LFB
- LFM/LFMD
- ICAL/CCV
- Control Charts
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency



TDEC - Fleming Training Center

21

Ammonia SM4500-NH3 D -1997

- Demonstration of Capability (DOC)
 - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
 - No limits listed for ammonia
 - Real people language: each operator running this test need to analyze 4 samples of an Ammonia Standard at a concentration around 1.0 mg/L.
 - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
 - Recommend backup analyst do this once a year.

TDEC - Fleming Training Center

22

Ammonia SM4500-NH3 D -1997

- MDL- Estimated Detection Level=0.03mg/L
 - From SM 1030 C.
 - $0.03\text{mg/L} \times 5 = 0.15 \text{ mg/L} \sim \text{MDL}$
 - Make a 0.15mg/L standard
 - Analyze 7 portions over ≥ 3 days
 - Calculate standard deviation (s)
 - $n1 \Sigma + n2 \Sigma + n3 \Sigma + \dots + n7 \Sigma + 2^{\text{nd}} \sigma_{xn} = s$
 - $s \times 3.14 = \text{MDL}$

TDEC - Fleming Training Center

23

Ammonia SM4500-NH3 D -1997

- Method Blank
 - Real people language: analyze distilled water as a sample by going through distillation (if you still distill samples) and using ISA (ion strength adjuster)
 - Target value is less than MDL (reporting limit)
 - Run on a 5% basis, one for every 20 samples
- Laboratory Fortified Blank
 - Real people language: analyze an ammonia standard at a concentration around 5 mg/L
 - Run on a 5% basis (see batch size for more information).

TDEC - Fleming Training Center

24

Ammonia SM4500-NH3 D -1997

- Lab fortified matrix and duplicate (spike& spike dup)
- Real people language – add a known amount of ammonia to a sample and expect that amount to be added to your sample concentration and repeat process for LFMD.
 - Run on a 5% basis (see batch size for more information).
 - Calculate RPD between spiked sample and spiked duplicate, target value should be close to the first value and have a small RPD (less than 20%).
 - **2014 Update** - Spike volume should be less than 1% of the volume.
 - Example: spike with 1 mL of 1000 mg/L into 100 mL sample will equal a 10 mg/L increase in ammonia concentration.

TDEC - Fleming Training Center

25

Ammonia SM4500-NH3 D -1997

- Initial Calibration
 - Standards that bracket the expected concentrations
 - Standards should not exceed an order of magnitude such as 1,10,100,1000
 - Real people language: calibrate probe daily (day of) with at least 3 standards
 - **2014 Update** – analyze a 10 mg/L standard as a sample after calibration and before samples to verify initial calibration (ICV)
- Calibration Verification
 - Real people language: analyze 10 mg/L at the end of samples daily (day of) to verify calibration is still valid

TDEC - Fleming Training Center

26

Ammonia SM4500-NH3 D -1997

- **2014 Update** - Create and maintain control charts if you have 20-30 data points within 90 days.
 - If you do not meet the above criteria, follow QC Acceptance Criteria below.
 - Blanks < MDL
 - LFB \pm 15%
 - ICV/CCV \pm 10%
 - LFM/LFMD \pm 20%
 - RPD < 20%
 - Reporting limit = MDL

TDEC - Fleming Training Center

27

Ammonia Nitrogen Procedure

1) Distillation

* Prepare Solutions:

- * Sodium Hydroxide, 6N – Dissolve 24 g of Sodium Hydroxide (NaOH) in 75 mL of ammonia-free distilled water and bring to 100 mL.
- * Boric Acid Solution - Dissolve 20 g boric acid in about 500 mL ammonia-free distilled water in a 500 mL beaker. Add this to a 1000 mL volumetric flask and dilute to 1000 mL. ****Don't need if running probe method.****
- * Borate Buffer Solution – Dissolve 9.5 g sodium borate in 500 mL of ammonia-free distilled water in a 500 mL beaker. Add this to a 1000 mL volumetric flask. Using a 100 mL graduated cylinder, add 88 mL of 0.1N NaOH to the volumetric flask. Mix well and dilute to 1000 mL.
- * Sulfuric Acid, 0.04N – Dilute 1.0 mL conc. H₂SO₄ to 1L.

* Prepare equipment:

- * Add 500 mL of ammonia-free distilled water to a 1000 mL beaker containing a stir bar. Add 20 mL of borate buffer to beaker. Adjust the pH to 9.5 with 6N NaOH.
- * Transfer the beaker contents to each distillation flask. Add 5 boiling beads to each flask. Connect all parts of the distillation apparatus.
- * Turn the cold water and heat elements on. Collect 50 mL portions of distillate in each of two 150 mL beakers. Test for ammonia by using ammonia test strips in each beaker. Discard water. Continue until distillate shows no trace of ammonia on test strip. Turn off the apparatus and allow flasks to cool.
- * Pour out contents of each distillation flask, collecting boiling beads in a Buchner funnel. Equipment is now ammonia-free.

- * Prepare samples:
 - * Add boiling beads back to each flask. Measure 500 mL of each sample (***primary clarifier effluent*** and ***plant effluent***) into 1000 mL beakers containing stir bars. Add 25 mL borate buffer to beaker with sample. Adjust pH to 9.5 with 6N NaOH. Add the sample to distillation flask.
 - * ***Note: we will be using raw intake instead of primary clarifier effluent from next door.***
- * Sample Distillation:
 - * Pour 50 mL 0.04 Sulfuric acid to one 500 mL glass stoppered Erlenmeyer flask which are marked at 250 mL (use a graduate cylinder, measure 250 mL and mark flask at water level)
 - * Submerge the delivery tube in the sulfuric acid in the flask.
 - * Distill the samples at the rate of 6-10 mL/min with delivery tube submerged in sulfuric acid (should take 20 – 33 minutes once distillation starts).
 - * Collect distillate up to the 250 mL mark (this is 200 mL distillate and 50 mL sulfuric acid)
 - * Set the distillate aside and continue distillation into a 150 mL waste beaker for 3 minutes to cleanse the tube and condenser. Turn off heat element.
 - * When cool, add each distillate to a 500 mL volumetric flask. Label the flasks as appropriate. Dilute to the 500 mL mark with ammonia-free distilled water.

Nitrogen, Ammonia

DOC316.53.01235

USEPA¹ Direct Measurement ISE Method²

Method 10001

0.1 to 10.0 mg/L NH₃-N

ISE Electrode

Scope and Application: For wastewater

¹ USEPA Accepted for reporting wastewater analyses² Adapted from *Standard Methods for the Examination of Water and Wastewater*, 20th Edition, Method 4500NH3E (with distillation). Manual distillation may not be required if comparability data on representative samples in company files show the distillation is not necessary. Manual distillation will be required to resolve any controversies.

! Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Meter	Electrode
sens \dot{i} on™ 4 meters	5192700
sens \dot{i} on™ 2 meters	5192700

Before starting the test:

Refer to the meter manual for meter operation. Refer to electrode manual for electrode maintenance and care.

Prepare the electrode. Refer to *New electrodes or electrodes stored more than 7 days* and *Electrodes stored 1 to 7 days* for more information.

After every hour of continuous use, place the electrode in the storage solution for 10 minutes to thoroughly recondition. Check with a 10 mg/L NH₃-N standard for accuracy and calibrate if necessary.

At high pH, ammonia solutions lose ammonia to the atmosphere, lowering the concentration. It is important to take measurements as soon as possible after the solution is basic. For most wastewater samples, 1 mL of 10 N NaOH (or equivalent ISA) is sufficient to increase the pH above 11. If in doubt, check the pH with pH paper and add additional NaOH in 0.1 mL increments until the pH exceeds 11.

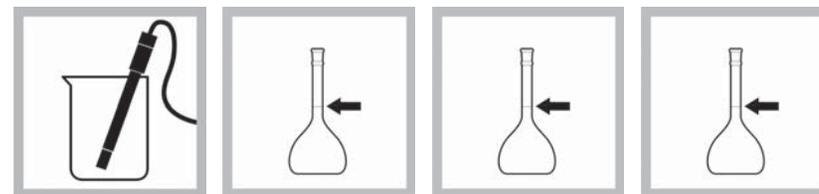
Nitrogen, Ammonia

Collect the following items:

Description	Quantity
Ammonia electrode filling solution	varies
Ammonia electrode storage solution	20 mL
Ammonia nitrogen standard, 100 mg/L NH ₃ -N	varies
Water, deionized	100 mL
Ammonia ISA solution	2 mL per 100 mL sample
Ammonia electrode, combination BNC	1
Beaker, 150 mL, polypropylene	4
Wash bottle	1
TenSette® pipet, 1.0–10.0 mL	1
sens \dot{i} on 4 laboratory pH/ISE meter or sension 2 portable pH/SE meter	1
Stir bar, 22.2 x 4.76 mm (7/16 x 3/16 in.)	4
Stirrer, electromagnetic, with stand and stir bar	1
25 mL Class A volumetric pipet	1
250 mL Class A volumetric flask	3

See *Consumables and replacement items* for reorder information.

Nitrogen, Ammonia in wastewater method



1. Rinse the electrode with deionized water. Place it in the Ammonia ISE storage solution with the ammonia membrane sensor module on to condition for at least 15 minutes.
2. During the conditioning period prepare three standards. Make a 10-mg/L NH₃-N standard by pipeting 25 mL of 100-mg/L NH₃-N Standard into a 250-mL volumetric flask. Dilute to the mark with ammonia-free deionized water, stopper and thoroughly mix.
3. Prepare a 1.0-mg/L NH₃-N standard by pipeting 25 mL of the 10-mg/L standard into a 250-mL volumetric flask. Dilute to the mark with ammonia-free deionized water, stopper and thoroughly mix.
4. Prepare a 0.1-mg/L NH₃-N standard by pipeting 25 mL of the 1.0-mg/L NH₃-N standard into a 250-mL volumetric flask. Dilute to the mark with ammonia-free deionized water, stopper and thoroughly mix.

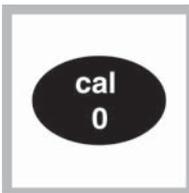
Nitrogen, Ammonia in wastewater method (continued)



5. Connect the Ammonia ISE to the BNC connector on the pH/ISE meter. Verify that BNC is selected in Setup 1 of the Setup menu.



6. Turn the meter on. Press **ISE/MV** until the display shows mg/L or other chosen concentration units.



7. Press **CAL**. Use the **ARROW** keys to select the desired units. Press **ENTER** and accept the units.



8. Transfer 100 mL of the 0.1-mg/L $\text{NH}_3\text{-N}$ standard to a 150-mL beaker. Add a stir bar to the beaker. Put the beaker on a magnetic stirrer and stir at a moderate rate.



9. Remove the electrode from the storage solution. Rinse it with deionized water and blot dry. Put the electrode into the 0.1-mg/L $\text{NH}_3\text{-N}$ standard. Make sure no air bubbles are trapped under the tip of the electrode.



10. Pipet 2.0 mL of Ammonia ISA Solution into the standard. Immediately proceed to the next step.



11. Press **READ**. The display will show the value from the previous calibration. Accept the numerical value or use the number keys to change the value to match the concentration of the standard, then press **ENTER** to accept the change.

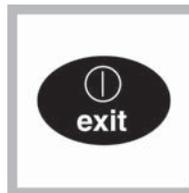


12. The display will show **Stabilizing...** until the reading is stable. The display will show **Standard 2** and _____ or the value of standard 2 from the previous calibration.

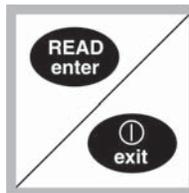
Nitrogen, Ammonia in wastewater method (continued)



13. Rinse the electrode with deionized water. Place it in the storage solution for one minute. Repeat steps 8–13 for substituting the 1.0- and 10-mg/L standards.



14. After the last standard is measured, press **EXIT**.



15. The display will show **Store?**. Press **ENTER** to store the calibration or **EXIT** to leave the calibration mode without storing the calibration values.



16. Press **REVIEW**. Use the Up arrow key to scroll to the second slope value. It should be $-57 \pm 3 \text{ mV/decade}$. If the slope is not $-57 \pm 3 \text{ mV/decade}$, recalibrate the electrode. If the slope is still incorrect after recalibration, replace the ammonia membrane sensor module. Press **EXIT** to return to measurement mode.



17. Remove the electrode from the last standard. Rinse it with deionized water and place it in the storage solution.



18. Transfer 100 mL of sample to a 150-mL beaker. Add a stir bar to the beaker. Put the beaker on a magnetic stirrer and stir at a moderate rate.



19. Remove the electrode from the storage solution. Rinse with deionized water and blot dry. Put the electrode into the sample.



20. Pipet 2.0 mL of Ammonia ISA solution into the sample and proceed immediately to the next step.

Nitrogen, Ammonia in wastewater method (continued)



21. Press **READ**. The display will show Stabilizing... until the reading is stable. Record or store the measurement value.

Repeat steps 17–21 for other samples.

Stabilization times will take longer for lower concentrations. A slow downward drift in concentration indicates probable loss of ammonia to the atmosphere. Record the highest value that is stable.

Calibration

Prepare ammonia standard working solutions of 10.0, 1.0 and 0.1 mg/L ammonia nitrogen from a 100-mg/L stock solution. Prepare the standards daily before use. Higher or lower concentration ranges (0.05–1400 mg/L $\text{NH}_3\text{-N}$) can be obtained by calibrating the meter with different standard solutions.

Electrode preparation

New electrodes or electrodes stored more than 7 days

Before using a new Ammonia Electrode or an electrode that has been stored dry, remove the protective cap from the end.

1. Unscrew the top cap. Carefully remove the internal glass electrode from the outer body. A white membrane is mounted at the tip of the outer body.
2. Fill the outer body with 3.5 mL of Internal Fill Solution.
3. Rinse the internal glass electrode with deionized water. Blot dry. Return the electrode to the filled outer body. Make sure that the key pin at the top of the internal glass electrode is seated in the slot at the top of the outer body.
4. Reinstall the threaded top cap onto the top of the ammonia electrode body. Finger-tighten the cap until snug. **Do not over-tighten.**

Nitrogen, Ammonia

5. Hold the fully assembled electrode securely by one end and shake the electrode with an abrupt downward motion (like shaking the mercury down in a thermometer) to remove bubbles.
6. Place the assembled electrode into the Ammonia Electrode Storage Solution or 1000 mg/L Ammonia Standard for at least 60 minutes.

Electrodes stored 1 to 7 days

- Keep the electrode in 1000 mg/L ammonia standard without Ionic Strength Adjustor (ISA).
- Alternatively, keep the electrode in the Ammonia Electrode Storage Solution.
- Never let the membrane dry out. Cover the storage beaker and electrode body with Parafilm® to prevent solution evaporation.

Electrodes stored between samples

Place the electrode in Ammonia Electrode Storage Solution for at least one minute to initialize the electrode for the next measurement.

Interferences

Distillation prior to ammonia analysis removes all inorganic interferences that complex ammonia.

Table 2 Interfering substances

Interfering substance	Interference level
Amines	Volatile low molecular weight gives a positive interference
Mercury	Complexes with ammonia
Silver	Complexes with ammonia

Sample collection, preservation and storage

- Collect samples in glass or plastic containers of convenient size. Clean new bottles by washing with deionized or distilled water. Fill the sample bottle completely and stopper immediately. Analyze the sample as soon as possible.
- Ammonia may be lost more quickly from samples at temperatures above 50 °C, so it is important to collect samples at less than 40 °C or use a cooling coil between the bottle and sampling point if necessary.
- If chlorine is present, treat the sample immediately with sodium thiosulfate. Add one drop of 0.1 N Sodium Thiosulfate Standard Solution for each 0.3 mg of chlorine present in a one liter sample.
- If prompt analysis is not possible, preserve the sample with 0.8 mL of concentrated sulfuric acid per liter. Use a sension pH meter to be sure the pH of the preserved sample is between 1.5 and 2. Some wastewater samples may require more sulfuric acid to achieve this pH. Store the sample at 4 °C. Samples preserved in this manner may be stored up to 28 days.
- Before analysis, neutralize the sample to pH 7 with 5 N sodium hydroxide. Do not let the pH go above 10. Correct the test results for the volume addition.
- Do not use mercuric chloride as a preservative because ammonia complexes with mercuric ions.

Nitrogen, Ammonia

Accuracy check

Standard additions method (sample spike)

To verify measurement accuracy, perform a standard addition spike on the sample. The spike should roughly double the measured concentration without significantly diluting the sample.

To perform a standard addition sample:

1. Use the *Spike volumes for standard additions* table to determine the concentration and volume of standard to spike the sample. The volume of sample transferred must be accurate.
2. Add the amount and concentration specified in the *Spike volumes for standard additions* table to the 100 mL of sample.
3. After adding the standard, proceed with the calculations. Results from 90-110% recovery are typically considered acceptable. Calculate percent recovery as follows:

$$\% \text{ Recovery} = \frac{100(X_s - X_u)}{K}$$

Where:

X_s = measured value for spiked sample in mg/L

X_u = measured value for unspiked sample adjusted for dilution by the spike, in mg/L

K = known value of the spike in the sample in mg/L

Calculations

$$1. X_u = \frac{X_1 \times V_u}{V_u + V}$$

Where:

X_1 = measured value of unspiked sample in mg/L

V_u = volume of separate unspiked portion in mL

V = volume of spike in mL

$$2. K = \frac{C \times V}{V_u + V}$$

Where:

C = concentration of standard used in spike in mg/L

V = volume of spike in mL

V_u = volume of separate portion before spike in mL

$$3. \text{ Final calculation plugging in } X_u \text{ and } K: \% \text{ Recovery} = \frac{100(X_s - X_u)}{K}$$

Nitrogen, Ammonia

Example:

A sample was analyzed and read 5.0 mg/L $\text{NH}_3\text{-N}$. As directed in the *Spike volumes for standard additions* table, a 4.0-mL spike of 100-mg/L $\text{NH}_3\text{-N}$ standard was added to another 100-mL sample, giving a final standard addition result of 8.75 mg/L.

Calculate the percent recovery as follows:

$$1. X_u = \frac{5.0 \text{ mg/L} \times 100 \text{ mL}}{100 \text{ mL} + 4 \text{ mL}} = 4.81 \text{ mg/L}$$

$$2. K = \frac{100 \text{ mg/L} \times 4 \text{ mL}}{100 \text{ mL} + 4 \text{ mL}} = 3.85 \text{ mg/L}$$

$$3. \%R = \frac{100 \times (X_s - X_u)}{K} = \frac{100 \times (8.75 - 4.81)}{3.85} = 102.3 \% \text{ Recovery}$$

Table 3 Spike volumes for standard additions

Measured Sample Concentration (mg/L)	Measured Sample Volume (mL)	Standard Concentration (mg/L)	Standard Volume (mL)
0.1–0.3	100	100	0.2
0.3–0.5	100	100	0.4
0.5–0.7	100	100	0.6
0.7–0.9	100	100	0.8
0.9–1.1	100	100	1.0
1.0–3.0	100	100	2.0
3.0–6.0	100	100	4.0
6.0–10.0	100	100	8.0

Method performance

Instrument	Standard	Precision 95% Confidence Limits of Distribution
<i>sensIon</i> 4 ¹	0.80 mg/L	0.78–0.82 mg/L
<i>sensIon</i> 2 ¹		

¹ With a default stabilization criteria of 0.5 mV/min.

Summary of method

The ammonia electrode measures ammonia gas or ammonium ions in aqueous solutions that have been converted to gas by the addition of a strong base. The electrode is a complete electrochemical cell consisting of a glass pH electrode and a reference electrode.

The gas-permeable membrane separates the sample from a thin layer of electrolyte that is pressed between the pH bulb and the membrane. At high pH, ammonium ion is converted to ammonia gas.

The gas diffuses through the membrane and causes a pH change in the thin layer of electrolyte. The potential across the pH glass changes as a result of the pH change and the electrode measures the change in potential. The measured pH change is proportional to the ammonia concentration in the solution.

Nitrogen, Ammonia

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode Filling Solution	varies	60 mL	4447226
Ammonia Electrode Storage Solution	20 mL	500 mL	2541249
Ammonia Nitrogen Standard, 100 mg/L NH ₃ -N	100 mL	500 mL	2406549
Ammonia ISA Solution	2 mL/100 mL sample	500 mL	2824349
Water, deionized	100 mL	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Ammonia Electrode	1	each	5192700
Beaker, 150 mL, polypropylene	4	each	108044
Bottle, wash, 500 mL	1	each	62011
Flask, volumetric, Class A, 250 mL	3	each	1457446
<i>sens</i> ion 4 Laboratory pH/ISE Meter or <i>sens</i> ion 2 pH/ISE (portable)	1	each	5177500
Stir Bar, 22.2 x 4.76 mm	4	each	4531500
TenSette® Pipet, 1.0–10.0 mL	1	each	1970010
Pipet tips for 1970010 TenSette Pipet	varies	50/pkg	2199796
Class A 25 mL volumetric pipet	1	each	1451540
Safety bulb pipet filler	1	each	1418900
Select one based on available voltage:			
Stirrer, electromagnetic 115 V, with stand and stir bar	1	each	4530001
Stirrer, electromagnetic 230 V, with stand and stir bar	1	each	4530002

Optional reagents

Description	Unit	Catalog number
Ammonia Nitrogen Standard Solution 1000 mg/L NH ₃ -N	1 L	2354153
pH Paper, pH 9.0-12.0	5 rolls/pkg	38533
Sulfuric Acid, concentrated	500 mL	97949

Nitrogen, Ammonia

Optional apparatus

Description	Unit	Catalog number
Air Gap Assembly	each	5025300
Ammonia Electrode Membrane Modules	4/pkg	5192711
Cylinder, graduated, glass	100 mL	50842
Electrode Washer	each	2704700
Pipet, Volumetric, Class A, 1.00 mL	each	1451535
TenSette® Pipet, 0.1–1.0 mL	each	1970001
Pipet tips for 1970001 TenSette Pipet	50/pkg	2185696
<i>sens</i> ion 2 Portable pH/ISE Meter	each	5172500

40 CFR 136 Table 1B says the approved methodology is manual distillation⁶ or gas diffusion (pH>11) followed by any of the following: Nesslerization, titration, electrode, manual phenate or automated phenate. Footnote 6 states: “Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary: however, manual distillation will be required to resolve any controversies. **In general, the analytical method should be consulted regarding the need for distillation.** If the method is not clear, the laboratory may compare a minimum of 9 different sample matrices to evaluate the need for distillation. For each matrix, a matrix spike and matrix spike duplicate are analyzed both with and without the distillation step. (A total of 36 samples, assuming 9 matrices). If results are comparable, the laboratory may dispense with the distillation step for future analysis. Comparable is defined as < 20% RPD for all tested matrices). Alternatively the two populations of spike recovery percentages may be compared using a recognized statistical test.”

Standard Methods

- 4500-NH₃ A.1 – In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater and good-quality nitrified wastewater effluent.
- 4500-NH₃ D.1.b. – Sample distillation is unnecessary.

Tennessee recommends that one sample is run yearly to compare the distilled and undistilled results and that the results are within 20% of each other.

- Note – if distilled sample and undistilled sample are below detection limit, you cannot calculate the percent difference.

Initial Demonstration of Capability (DOC)

- 1020 B. 1 – As a minimum, include a reagent blank and at least 4 LFBs at a concentration between 10 times the MDL and the midpoint of a calibration curve.
- 4020 B.1.a. - each analyst must run a known standard concentration at least four times and compare limits listed in the method.
- **Real people language – each operator running this test needs to calibrate the probe and analyze 4 samples of an NH₃ Standard at a concentration around 1.0 mg/L**
 - **Keep a folder for each analyst, keep a copy here**
 - **Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.**
 - **Recommend backup analyst do this once a year.**

Method Detection Limit (MDL)

- 1020 B. 4 – As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level (5 x 0.03 mg/L = 0.15 mg/L).
 - Ideally, prepare and analyze at least seven (7) portions of this solution over a 3-day period to ensure that the MDL determination is more representative of routine measurements as performed in the laboratory.
 - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD (relative standard deviation) values ≤ 20%.

- 4020 B.1.b. – Verify MDL at least **annually**.
 - Ideally use pooled data from several analysts rather than data from one analyst.
- **Real people language – have several operators, who run this test, analyze an NH₃ Standard at a concentration of 0.15 mg/L over several days with a total of at least 7 samples**
 - **Joe analyzes 3 samples on Monday**
 - **Bob analyzes 3 samples on Tuesday**
 - **Mary analyzes 3 samples on Wednesday**
- **Run this once a year**

Initial Calibration Verification (ICV)

- 1020 B.11.b. – Perform initial calibration using at least three concentrations of standards for linear curves.
- 4020 B.2.a. – Calibrate initially with at least one blank and three calibration standards.
 - The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
 - The back-calculated and true concentrations should agree within $\pm 10\%$.
- 4500-NH₃ D.4.a. – Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1 and 0.1 mg NH₃-N/L
- 4500-NH₃ D.4.b. – calibrate from lowest to highest concentration. Wait until the reading has stabilized (at least 2-3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH₃-N/L.
- 4500-NH₃ D.4.c. – If the electrode is functioning properly, a tenfold change of NH₃-N concentration produces a potential change of about 59 mV.
- **Real people language – calibrate according to manufacturer's instructions with at least 3 standards that will bracket your sample range daily (day of).**
- **Analyze a 10 mg/L standard as a sample after calibration and before samples to verify initial calibration (ICV)**

Method Blank – goes through distillation if you distill

- 1020 B.5.– A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure.
- 4020 B.2.d. – Include at least one method blank daily or with each **batch of 20** or fewer samples, whichever is more frequent.
 - If any method blank measurements are at or above the reporting level, take immediate corrective action.
- **Real people language – analyze distilled water as a sample by going through distillation (if you still distill samples) and using ISA (ion strength adjuster).**
 - **Target value is less than reporting limit**
 - **Reporting limit will be equal to your Method Detection Limit (MDL)**
 - **Run on a 5% basis (see batch size for more information).**

Laboratory Fortified Blank (LFB) – goes through distillation if you distill

- 1020 B.6.– A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added.

- Section 3
- Sample batch = 5% basis = 1 every 20 samples TDEC - Fleming Training Center
 - Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve.
 - 4020 B.2.e. – Calculate percent recovery, plot control charts and determine control limits (see Control Charts below)
 - **Real people language – analyze an NH₃ standard at a concentration of 5.0 mg/L**
 - **Run on a 5% basis (see batch size for more information).**

Duplicate

- NONE

Laboratory Fortified Matrix (LFM)/Laboratory Fortified Matrix Duplicate (LFMD) – goes through distillation if you distill

- 1020 B.7.– A laboratory fortified matrix (LFM) is an additional portion of a sample to which a known amount of the analyte of interest is added before sample preparation
 - The LFM is used to evaluate analyte recovery in a sample
 - Sample batch = 5% basis
 - Add a concentration that is at least 10 times the MRL (minimum reporting level), less than or equal to the midpoint of the calibration curve.
 - Preferably use the same concentration as the LFB
- 4020 B.2.g. – When appropriate for the analyte, include at least one LFM/LFMD daily or with each batch of 20 or fewer samples
 - Add a known concentration of analyte to a randomly selected routine sample
 - Calculate percent recovery and relative percent difference, plot control charts and determine control limits for spikes at different concentrations (see Control Charts below)
- **Real people language – add a known amount of ammonia to a sample and expect that amount to be added to your sample concentration and repeat process for LFMD.**
 - **Run on a 5% basis (see batch size for more information).**
 - **Calculate RPD between spiked sample and spiked duplicate, target value should be close to the first value and have a small RPD (less than 20%).**
 - **Spike volume should be less than 1% of the volume.**
 - **Example: spike with 1 mL of 1000 mg/L into 100 mL sample will equal a 10 mg/L increase in ammonia concentration.**

Continuing Calibration Verification (CCV)

- 1020 B.11.c. – Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
 - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- 4020.B.2.b. – Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
 - For the calibration verification to be valid, check standards must be within 10% of its true value
- **Real people language – analyze 10 mg/L at the end of all samples daily (day of).**

Ammonia

TDEC – Fleming Training Center

S. Pratt, January 2014



- **Real people language**
 - **Create and maintain control charts if you have 20-30 data points within 90 days.**
 - **If you do not meet the above criteria, follow QC Acceptance Criteria below.**

Corrective Action - 1020 B.5., B.8., & B.15.

QC Acceptance Criteria

- Blanks < MDL
- LFB \pm 15%
- ICV/CCV \pm 10%
- LFM/LFMD \pm 20%
- RPD < 20%
- Reporting limit = MDL

Batch Size

- For samples that need to be analyzed on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent) follow these criteria:
 - If a permit stated that 3 analyses per week, we would allow for a duplicate to be analyzed at least once per month.
 - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
 - If a permit stated 5 analyses per week, we would allow twice a month.
 - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!
 - If sampling only once a month, need to run QC once a month.

Calculations

- % Recovery for LFB
 - = $\frac{\text{LFB Result}}{\text{Expected Concentration}} \times 100\%$
- RPD – relative percent differences for duplicates and LFM/LFMD
 - = $\frac{\text{Difference between sample and duplicate}}{\text{Average of the sample and duplicate}} \times 100\%$
- % Recovery for LFM – when using less than or equal to 1% spike volume compared to sample volume
 - = $\frac{\text{LFM Result} - \text{Sample Result}}{\text{Actual Concentration of spike}} \times 100\%$

Section 4 Activated Sludge Process Control





PROCESS CONTROL TESTING

1

TDEC - Fleming Training Center

ACTIVATED SLUDGE PROCESS CONTROL

- o What is a process?
 - Continuing operation or development marked by a series of gradual changes that succeed one another in a relatively fixed way and lead toward a particular result or end.



2

TDEC - Fleming Training Center

ACTIVATED SLUDGE PROCESS CONTROL

- o What is the wastewater process result or end?
 - Clean Water
 - BOD removal
 - Solids removal
 - Good solids/liquid separation

3

TDEC - Fleming Training Center

TREATMENT PLANT PROCESSES

- o Flow Monitoring, Equalization
- o Screening
- o Grit and Grease Removal
- o Activated Sludge
 - Aeration Basin, Clarifier, RAS/WAS
- o Disinfection

4

TDEC - Fleming Training Center

W/W TREATMENT & MANUFACTURING

<ul style="list-style-type: none"> o Wastewater <ul style="list-style-type: none"> • One raw material • Treatment process <ul style="list-style-type: none"> o Several Steps gradually leading to finished products. • Finished products <ul style="list-style-type: none"> o Trash, Solid waste rules o Clean Water, Permit o Biosolids, 503 rule 	<ul style="list-style-type: none"> o Manufacturing <ul style="list-style-type: none"> • Many raw materials • Manufacturing process <ul style="list-style-type: none"> o Several Steps gradually leading to finished products. • Finished product <ul style="list-style-type: none"> o Sausage, cars, pencils; all of which have quality specifications
---	---

5

TDEC - Fleming Training Center

COMMON PROCESS CONTROL METHODS

- o How do you control your activated sludge process?
 - Human senses
 - o Visual appearance, odors
 - Process tests
 - o Flow, D.O., pH, temp., alkalinity, ORP, turbidity
 - o Settler, Sludge judge
 - o MLSS, MLVSS
 - o Centrifuge spins
 - o Microscopic evaluation
 - o Oxygen Uptake Rate, Specific Oxygen Uptake Rate

6

TDEC - Fleming Training Center

PROCESS CONTROL

- o Test performed in the aeration basin and or clarifier that indicate what the effluent quality will be when the water leaves the treatment plant.
- o There may be a significant time delay from the time water enters the aeration basin and effluent sampling or discharge.
- o Manufactures face similar quality challenge.

TDEC - Fleming Training Center

7

PROCESS CONTROL

- o What aerator test “now” will assure you of good effluent when that water reaches the effluent several hours latter?

TDEC - Fleming Training Center

8

PROCESS CONTROL

- o You choose the method that assures you that effluent will meet permit.
- o NPDES permit
 - Part II.A.4 Proper Operations and Maintenance
 - o “...adequate process controls...”
 - o Though almost hidden, this is a Permit requirement
- o Find a method that works for you and use it!

TDEC - Fleming Training Center

9

SENSORY PROCESS CONTROL

- o Odors
 - o Fresh plowed field
 - o Hog pen
- o Turbulence
 - o Boiling, Dead spots
- o Foam and Scum
 - o Fresh, crisp, light-colored foam
 - o Billowing white foam
 - o Thick, scummy, dark foam

TDEC - Fleming Training Center

10

SENSORY PROCESS CONTROL

- o Clarifier
 - Bulking, sludge quality
 - Billowing, hydraulic overload
 - Clumping, denitrification
 - Ashing/Pin Floc, old sludge
 - Straggler Floc, young sludge

TDEC - Fleming Training Center

11

PROCESS CONTROL

- o Flow Rates, accurate flow measurements of premier importance.
- o Locations
 - Influent Q
 - RAS, WAS, other
- o Dissolved oxygen
 - >0.5mg/L for BOD removal, >2.0 for Ammonia
 - Profiles-longitudinal, vertical
 - DO levels are relative to the oxygen demand

TDEC - Fleming Training Center

12

PROCESS CONTROL, CONTINUED

- pH, 5-9 for BOD, 6.5-8 for Ammonia
 - Indicator of toxicity
 - Indicator of nitrification problems
- Temperature, Above freezing for BOD, 25°C optimal for AM.
 - Use D.O. meter
 - Effects speed of bacterial metabolism, or perhaps no metabolism!

TDEC - Fleming Training Center

13

PROCESS CONTROL, CONTINUED

- Alkalinity, effluent >50mg/L for Ammonia removal
 - Necessary for complete nitrification
- ORP-Oxidation Reduction Potential, Redox
 - pH meter with ORP probe
 - Indicated the oxidative state of the solution
- Turbidity
 - Indicator of completeness of flocculation

TDEC - Fleming Training Center

14

PROCESS CONTROL, CONTINUED

- Settleometer
 - Use settleometer not graduated cylinder
 - Indicator of clarifier performance
 - How well the biomass- settles and compacts
- Sludge Judge, MLSS, MLVSS Centrifuge spins
 - Indicators of biomass inventory

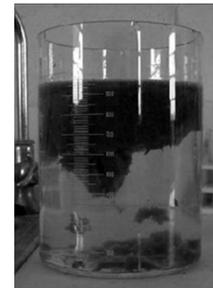


TDEC - Fleming Training Center

15

SETTLEOMETER

- Basic Process Control
- 5min, How fast sludge settles
- 30 min, How well sludge compacts
- Supernatant turbidity, how well sludge flocculates
- Denitrification



TDEC - Fleming Training Center

16

SETTLEOMETER

- Test Procedure:
 1. Initiate as soon as possible, lengthy delays may contribute to errors.
 2. Agitation of the sample may contribute to errors.
 3. Pour gently mixed sample into settleometer, filling settleometer to top 1000 ml/L mark.
 4. Mix sample in settleometer with wide paddle, rocking back and forth, not twisting.
 5. Stop motion in settleometer (hold paddle in place) resulting in sharp interface.
 6. Lift mixing paddle directly from settleometer and start timer.

TDEC - Fleming Training Center

17

SETTLEOMETER

- Test Procedure:
 7. Record actual time of test initiation, $t = 0$ in case of missed reading
 8. Observe first 5 minutes of test
 9. Record settled sludge volume at 5-minute intervals for the first thirty minutes of test (for SVI and ultimate compactness for fast settling sludges).
 10. Record settled sludge volumes at 10-minute intervals for the second thirty minutes of test (ultimate compactness for normal settling sludges).

TDEC - Fleming Training Center

18

SETTLEOMETER

- o Test Procedure - continued:
 11. For slow settling sludges, record settled sludge volumes at half hour intervals until ultimate compactness. Ultimate compactness is defined as the settled sludge volume at which settling and compacting has ceased in the settleometer. Knowledge of the ultimate compactness value is required for proper return sludge control of slow settling sludges.
 12. Record rise time. Rise time is defined as the interval required for the sludge to rise in the settleometer. Knowledge of the rise time changes is indicative of denitrification trends in secondary clarifiers.

19

TDEC - Fleming Training Center

SETTLEOMETER - DATA

- o Settled Sludge Volumes (SSV):
 - SSV5, SSV10, SSV15, SSV20, SSV25
 - SSV30 (SVI; fast settling sludge)
 - SSV40, SSV50
 - SSV60 (normal settling sludge)
 - SSV90, SSV120 (ultimate compactness; slow settling sludge)
- o Rise Time (denitrification of sludge)

20

TDEC - Fleming Training Center

SETTLEOMETER - DATA

- o May want to run a diluted sample with 50% effluent and 50% MLSS and compare to the undiluted sample.
- o If the diluted samples settle significantly quicker than the undiluted sample (especially during the first 10 minutes), the system contains too many solids and wasting should be increased.

21

TDEC - Fleming Training Center

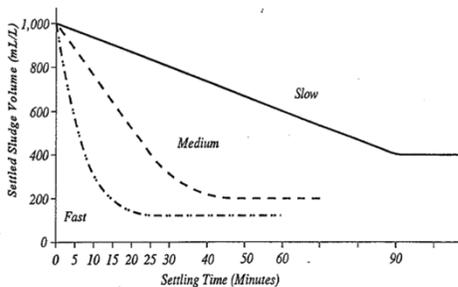
SETTLEOMETER - DATA

- o If the diluted sample settles at the same rate or only slightly faster than the undiluted sample, the MLSS is young and bulky.
 - This can be caused by excessive wasting
 - To correct, reduce wasting a little each day to develop a sludge that settles better.
- o If the sludge settles so slowly that you are losing solids in the effluent, you may need to add polymers to aid in settling.

22

TDEC - Fleming Training Center

SETTLEOMETER - DATA



23

TDEC - Fleming Training Center

CLARIFIER SOLIDS

- o Activated Sludge Process Control:
 - Mollory Cylinder or Settleometer
 - Glass or plastic flat bottom beaker
 - Sample (2 L) settles 30 min, then settled sludge volume read off side of cylinder
 - During test, rate and quality of settling are observed



24

TDEC - Fleming Training Center

SLUDGE VOLUME INDEX (SVI)

- Monitor activated sludge settling behavior for process control
- Calculation using MLSS (mg/L) and 30 min SSV (mL/L)
- $SVI = \frac{\text{mL/L after 30 min}}{MLSS \text{ mg/L}}(1,000)$
- Target 100, Range 50-150

TDEC - Fleming Training Center

25

SETTLED SLUDGE CONCENTRATION (SSC)



- Centrifuge test for sludge quality
- Aeration tank mixed liquor concentration (ATC)
- Tubes contain 10 mL
- Centrifuge 15 min at 3000 rpm

TDEC - Fleming Training Center

26

SETTLED SLUDGE CONCENTRATION (SSC)

- Read level of compacted solids. Multiply result by 10 to obtain sludge concentration or % solids (ATC)
- Can be used on return and waste sludges
- Calculate SSC for any SSV as:
 - $SSC = (1000)(ATC/SSV)$
- Plot settleable vs. centrifuge solids for quick estimate of settleable solids

TDEC - Fleming Training Center

27

BIOMASS INVENTORY

- Inventory of Biomass should answer three questions
 - How much sludge is in the system?
 - Where is it located?
 - How long has it been there?
- Experience has shown us certain sludge ages give us certain effluent qualities.
- With these answers, process control is easy

TDEC - Fleming Training Center

28

BIOMASS INVENTORY

- Thumb Rules
 - BOD Removal
 - MCRT, 0.5-1 Day
 - Ammonia Removal
 - MCRT 4-15 Days
- There are exceptions to all rules!!!



TDEC - Fleming Training Center

29

MICROSCOPIC EVALUATION

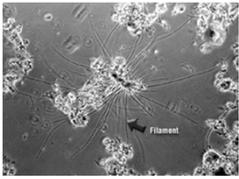
- Floc analysis, Jenkins' Book
 - General shape, size, dispersed cells
- Protozoan/ Metazoan counts
 - General indicator of sludge age
- Filaments
 - Abundance, inside/outside flock, bridging
 - Non-Phase microscope, ID- *Nocardia*, *Beggiatoa*
- Slime Bulking
 - India ink test

TDEC - Fleming Training Center

30

MICROGRAPH OF FLOC AND FILAMENTS

- Filamentous bacteria are not "floc formers" but are also of interest in WW treatment.
- Small amounts of them can improve floc structure, acting as a back bone, providing mass to help in settling after treatment.
- Large amounts can negatively affect performance of activated sludge systems by keeping floc apart and which makes it light and fluffy, therefore, not settling well.



TDEC - Fleming Training Center

31

PROTOZOA

- Single-celled animals that also reproduce by binary fission
- Have complex digestive systems that ingest organic matter, which they use as an energy and carbon source
- Protozoans are much larger than bacteria, their size ranges from 10-500 microns
- They are an important link in the activated sludge food chain because they consume bacteria to fill a large part of their nutritional needs.
 - This seems not only to remove excess bacteria from WW, but appears to stimulate the growth of healthy bacteria, which produce floc more quickly and aid in the clarification of the effluent
- Form cysts
- Beneficial in wastewater treatment
- Indicators of health of system

Examples:

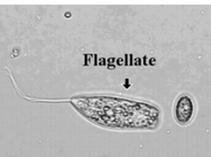
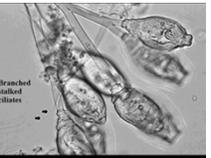
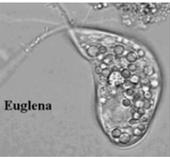
- Amoeba
- Free-Swimming Ciliates - Paramecium
- Crawling Ciliates
- Stalked Ciliates
- Suctoria

TDEC - Fleming Training Center

32

PROTOZOA FOUND IN THE ACTIVATED SLUDGE PROCESS

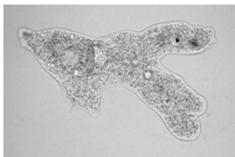
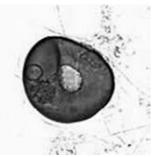
- Much less abundant than bacteria, but very important.
- Require DO
- Flagellate has a whip-like tail and competes with bacteria
- Stalked ciliates – as adults, attach to something; as a "baby", has little hairs (cilia) to move around and move water and food into "mouth"
- Euglena – has green algae in it that makes oxygen when the sun shines.

TDEC - Fleming Training Center

33

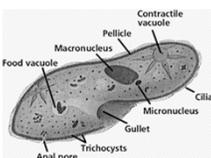
PROTOZOA – AMOEBIA

TDEC - Fleming Training Center

34

PROTOZOA – FREE SWIMMING CILIATE (PARAMECIUM)

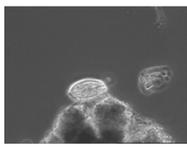



- Free swimming ciliates generally are younger biomass organisms but are common in many plants.
- Cilia covers entire shape
- Sufficient D.O.
- Asexually & Sexually
- Paramecium- 4.7 hours growth rate

TDEC - Fleming Training Center

35

PROTOZOA – CRAWLING CILIATES

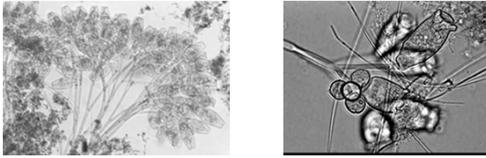



- Resemble crabs or ladybugs
- May have some cilia but majority of body does not contain any
- Croppers of biomass
- Cirri (A bundle or tuft of cilia serving as foot or tentacle in certain ciliate protozoa) are 4-5 cilia fused together
- Very efficient feeders

TDEC - Fleming Training Center

36

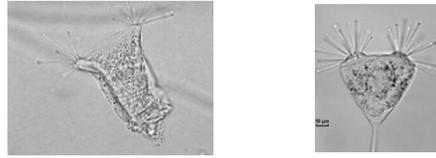
PROTOZOA – STALKED CILIATES



- They feed by drawing cells into their “mouth” with small cilia that create a visible twirling motion in the sample.
- Can be sessile or colonial
- Length of stalk indicates age
- Some will have a myoneme (contractile muscle fiber with in stalk)
- Some species will produce a daughter cell which resembles a free-swimming ciliate
- Size of oral opening may indicate health of system / more bacteria smaller opening and less bacteria larger opening
- Single (vorticella) vs colonial (epistylis) does not mean one is better than other, they are all individual species and grow based on the environment

37

PROTOZOA – SUCTORIA

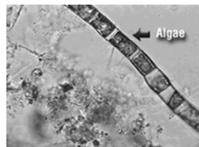


- These are the true vampires of the wastewater world
- Tentacles may recoil in presence of increased ammonia
- Some will have a stalk and others may not

38

FUNGI AND ALGAE

- Fungi
 - Soil organisms
 - Degrade dead organic matter (saprophytic)
- Algae
 - Photosynthetic
 - Eutrophication can cause algal blooms in receiving streams
 - Key in operation of wastewater ponds: produce oxygen needed by bacteria
 - Nuisance in clarifiers, basins, etc.



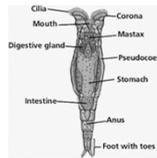
39

METAZOA

- Multi-cellular animals
- Multicellular
- Slower growing
- Typically larger than protozoa
- Sexual and asexual reproduction
- Heterotrophic
- All are motile
 - Unless there has been an upset to the plant
- Examples:
 - Rotifer
 - Water Mite
 - Water Bear
 - Nematodes
 - Ostracods

40

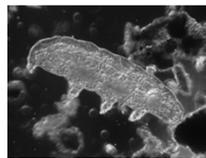
METAZOA – ROTIFER



- Simple multi-celled organisms
- Need aerobic environment
- Consume solid food including bacteria
- In lagoons, they eat lots of algae
- Means happy, healthy population
- Over 80% are female
- Longer Sludge age
- Low BOD, Sufficient D.O.
- Tardigrade food*
- Some move like snails others resemble free-swimming ciliates

41

METAZOA – WATER BEAR (TARDIGRADE)

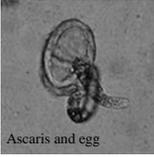


- Old sludge organism
- Feeds on smaller protozoa
- Does not like ammonia
 - Not found in presence of ammonia above 5ppm
- Extremely aerobic
- 8 legs- with 2 claws on each for holding
 - Prefer rotifers as a food source
- Water bears are typically not seen in industrial waste treatment systems
- They have been sent to space as part of the NASA program

42

METAZOA – NEMATODE

- o Multicellular organisms
- o Diseases (tapeworms, roundworms)
- o Beneficial in trickling filters (increase air penetration in biofilm and help in sloughing)



Ascaris and egg

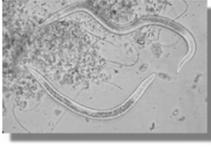
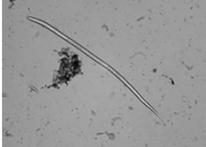


Trickling filter

TDEC - Fleming Training Center

43

METAZOA – NEMATODE

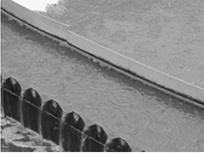



- o Aquatic earthworms.
- o Fast moving.
- o The poke around the floc.
- o Older sludge organisms that reproduce slowly.

TDEC - Fleming Training Center

44

METAZOA – BRISTLE WORM

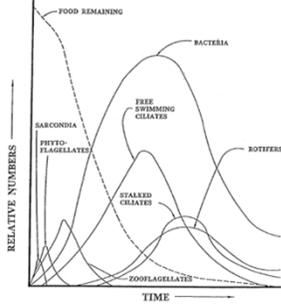



- o Aquatic earthworm
- o They eat bacteria and protozoa.
- o They are relative active. They have red spot that are not visible here but can turn biomass red colored.
- o They have the capacity to make your biomass disappear.

TDEC - Fleming Training Center

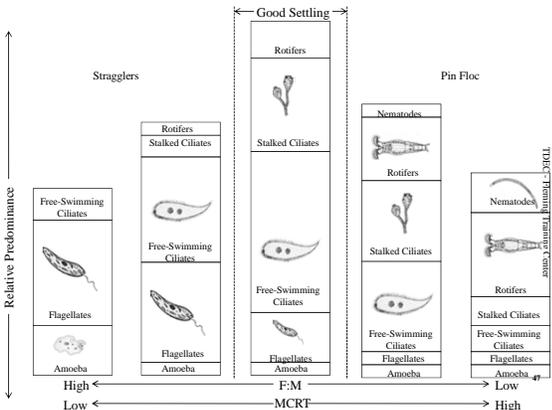
45

BACTERIA POPULATION VS. SLUDGE AGE



TDEC - Fleming Training Center

46



TDEC - Fleming Training Center

47

MICROORGANISMS PREDOMINANCE

- o If conventional plant and you start to see more rotifers and less free-swimming ciliates, you need to increase wasting to make old sludge go away/
- o If extended aeration plant and you have pin floc and nematodes, you are holding your sludge too long.

TDEC - Fleming Training Center

48

OXYGEN UPTAKE RATE

- Rate at which microorganisms use oxygen
 - How fast the biomass or bugs are eating, growing and reproducing or more scientifically, metabolizing the available substrate
 - Indicator of toxicity
 - Indicator of food abundance or ease of metabolism
- OUR varies with solids concentration
- SOUR accounts for solids variation
 - Commonly called Respiration Rate

TDEC - Fleming Training Center

49

OUR AND SOUR

- If the standard tests such as DO, pH, temperature, odor and appearance show differences from the normal, the effect of those differences to the biomass maybe indicated by an OUR or SOUR test.
- Changes could be due to industrial discharges both intentional and unintentional, illegal discharges to the collection system from pumpers to terrorist.

TDEC - Fleming Training Center

50

SOUR VALUES

- SOUR $>20\text{mgO}_2/\text{hr/gm MLVSS}$
 - Logarithmic growth, Flagellates, dispersed flock
 - Settling Slow $\text{SSV}_5 > 750\text{cc/L}$
 - SOUR $12\text{-}20\text{mgO}_2/\text{hr/gm MLVSS}$
 - Declining growth, Ciliates, Flocks forming
 - Settling normal $\text{SSV}_5 = 600\text{-}750\text{cc/L}$
 - Sour $<12\text{mgO}_2/\text{hr/gm MLVSS}$
 - Endogenous Respiration, Rotifers and higher life
 - Pin Flock
 - Settling Fast, $\text{SSV}_5 < 600\text{cc/L}$
- Remember the growth graph

TDEC - Fleming Training Center

51

SOUR VALUES

- By conducting background tests on your aeration basin, operators will generate historic data that will show what a “normal” SOUR level is for your facility
 - If a test value dramatically changes from normal, suspect a change in the influent or biomass characteristics

TDEC - Fleming Training Center

52

SOUR TEST METHOD – SM 2710

- In order to have results that reflect true aeration basin conditions analyze samples without delay.
- If DO levels in the sample are low (SM states $\leq 2.0\text{ mg/L}$), manually aerate the sample.
- DO values in the sample at the end of the test should be above 1.0 mg/L , a number which is also used in BOD test rules



TDEC - Fleming Training Center

53

LONG TERM PROCESS CONTROL

- F:M
- Food to Microorganism Ratio
- lbs. of Raw BOD
lbs. of MLVSS
- Even bugs want a adequate diet.
- Always at least 5 days late.



TDEC - Fleming Training Center

54

PROCESS CONTROL

- Human senses
 - Visual appearance, odors
- Process tests
 - Flow, D.O., pH, temp., alkalinity, ORP, turbidity
 - Settrometer, Sludge judge
 - MLSS, MLVSS
 - Centrifuge spins
 - Microscopic evaluation
 - Oxygen Uptake Rate, Specific Oxygen Uptake Rate

TDEC - Fleming Training Center

55

PROCESS CONTROL

- Choose a method that works for you.
- Collect the Data.
 - Data is the voice of the process.
- Use the Data!
- Make decisions based on the data!
- Graph the Data!
 - Picture of the numbers, picture of the process.

TDEC - Fleming Training Center

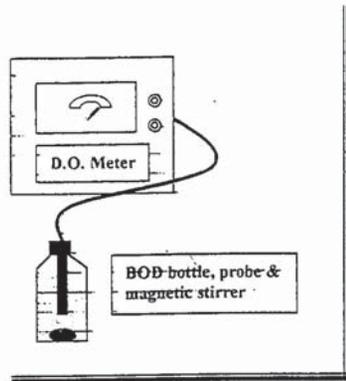
56

PROCESS CONTROL

- Find a method that works for you
- Use it!
 - Meet your Permit
 - Do better than your Permit
 - Operate at a lower cost
 - Become a better operator

TDEC - Fleming Training Center

57



OXYGEN UPTAKE TEST

I. Test Significance

O₂ uptake rate (or OUR) is the only quick test that an operator has at his disposal that allows him to assess feed acceptability as well as sludge quality. OUR is a simple test which can be run by an operator and it does not require elaborate lab skills.

II. Test Samples

- Mixed liquor (aeration tank effluent): This sample is the only sample referenced in Standard Methods, and consequently few individuals are familiar with the FED/UNFED OUR technique.
- Primary Effluent (FED mixture).
- Secondary Effluent (UNFED mixture): Unchlorinated.
- Return Sludge - Aerate as soon as possible after collection.

III. Test Frequency

- Once per day (during peak flow) to assess feed acceptability in large plants with industrial contribution. Perhaps even more frequently if plant has history of upsets.
- Once per week to assess sludge quality in small plants with no industry.

IV. Test Equipment

- Electronic DO analyzer w/bottle probe.
- Standard (300 ml.) BOD bottles.
- 250 ml. graduated cylinder.
- Magnetic mixer w/1 to 1 1/2" stirring bar.
- Sample containers.
- Tapered PVC (3/4" Pipe) Sleeve.
- Timer.
- Centrifuge.
- Centrifuge Tubes.

V. Test Procedure

Of course, OUR can be tested on the mixed liquor sample, but samples can be mixed together (Primary + Return = FED; Secondary + Return = UNFED) to assess activity after the return sludge is mixed with the incoming feed (FED). Additionally, an approximation of the oxygen used by the sludge after it is stabilized in the aeration basin and settled in the clarifier can be made (UNFED). Both samples are mixed such that the concentration approximates that of the mixed liquor.

Volume of sludge by flow measurements:

- $RSV = (AV - RSP) / (IFV - RSP)$.
- Where:
 - RSV = Return Sludge Volume (ml.).
 - AV = BOD Bottle Volume of 300 ml.
 - IFV = Influent Flow Percentage of 100 % (decimal).
 - RSP = Return Sludge Percentage of Influent Flow (decimal).

Note: Because of lags in the plant due to meter locations, tanks and detention times this technique has sometimes given erroneous results.

Volume of sludge by centrifuge:

- $RSV = (ATC/RSC) \times \text{BOD Bottle Volume of 300 ml.}$
- Where:
 - ATC = Aeration Tank Concentration by Volume (%)
 - RSC = Return Sludge Concentration by Volume (%)

**Examples: RSP = 40% influent flow
ATC = 3.0% RSC = 10.5%**

- By flow: $RSV = (300 \times .40) / (1.0 + .40) = 86 \text{ ml.}$
 - By centrifuge: $RSV = (3.0/10.5) \times 300 = 86 \text{ ml.}$
1. Calibrate DO meter.
 2. Mix and pour RSV into BOD bottle.
 3. Fill remainder of bottle (into neck to prevent air bubbles) with:
 - Primary effluent for FED sample.
 - Secondary effluent for UNFED sample.
 4. Insert Tapered PVC Sleeve.
 5. Place empty BOD bottle on top (ala Sludge Cocktail).
 6. Aerate sample by transferring contents from one bottle to another and agitating.
 7. Place back on magnetic mixer and remove bottle and PVC sleeve allowing any entrained air to escape.
 8. Insert magnetic stirring bar and DO electrode assembly.
 9. Adjust magnetic mixer to speed sufficient to maintain solids in suspension. Usually the mixer on the probe assembly is sufficient to suspend solids near the top of the bottle, but not always throughout the sample.

VI. Data Collection

- Prepare data record sheet including the following information (using centrifuge):

Date _____ Time _____ Temp. (°C) _____
RSC _____ ATC _____ RSV _____

Minutes	DO (mg./L.)	▲ DO mg./L./min.
0 (start)		
1		
2		
3		
4		
5		
6		

Notice that RSP may be substituted for RSC and ATC if flows are used. Also, time intervals may

require adjustment to half minutes for rapid OUR samples (e.g. FED). Adjust Time (min.) column to 0, 0.5, 1.0, 1.5, 2.0, etc.

- Observe and record initial temperature and DO, start the timer and observe and record the DO precisely at one-minute intervals. The DO meter should remain on during this phase so that the rate of change can be observed and half-minute readings can be taken if required.
- Record on data sheet until at least three (3) consistent ▲ DO readings are obtained.
- Record the final temperature. Caution should be taken that a hot-plate/magnetic mixer is not used with heat-on, as results will be extremely erroneous.
- Pour a sample for the test mixture into the centrifuge tube and test to check concentration. It should be very close to ATC.

VII. Special Variations or Applications of Test

Mixed liquor (Standard Methods)

Stabilization of the waste, as it leaves the aeration can be determined.

Stabilization Time Test

1. Reflects sludge activity during the aeration cycle.
2. Indicates events in progression after feeding, e.g.
 - possible lags.
 - irregular progression or phases.
 - extended time requirements.
 - excessive peak OUR.
3. As stabilization time proceeds.
 - OUR decreases.
 - flocculation tendencies increase.
 - settling rate increases.
 - solids/liquid separation improves (within limits).
4. A curve depicting the stabilization process can be drawn from the data gathered in this simple test.

Procedure.

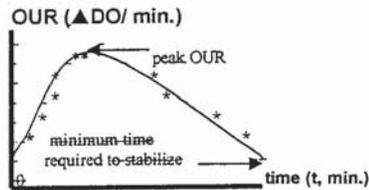
1. determine UNFED - OUR

2. mix and aerate the Return Sludge and Primary Effluent in a small tank (6 liters or more) in the same proportions as those that exist in the aeration basin using the ATC, RSC technique as before and knowing the final volume
3. (e.g. 6 L. = 20 BOD bottles).
4. upon initiation (t = 0) draw off 300 ml. bottle for OUR test.
5. perform OUR test and pour remainder of sample back into stabilization tank.

Data Collection.

- repeat OUR test every fifteen (15) minutes until OUR approaches UNFED - OUR.
- to develop the stabilization curve, plot OUR (Δ DO/min) vs. time (min.).

Example - Stabilization Test Curve



- Permits operator to determine if:
 1. adequate aeration time is available.
 2. excessive oxygen demands are being placed on system (which might require a change in operating mode, e.g. place another available aerator into operation).
 3. a new load is affecting system.

Normally, stabilization tests are developed only a few times a year of whenever the industrial contribution changes.

Aerobic Digesters (specific OUR or SOUR).

- SOUR expressed in mg. DO/gm. MLVSS/hr. may be used to determine the stability of aerobically digested sludges. Rates of 0.5 to 1.0 mg. DO/gm. MLVSS/hr. are normally associated with well-stabilized sludges.

BOD Estimates.

- An OUR test performed on undiluted (100%) unchlorinated secondary effluent can be correlated with BOD₅ data to obtain preliminary results on effluent quality. Although BOD is still required for reporting purposes, this can serve as an aid toward setting up dilutions for BOD measurement.
- Δ DO at 15-30 minute intervals gives an activity index related to effluent quality in less than four (4) hours.

VIII. Interpretation

Unfed Rate

UNFED follows the load during the day and week. For MLSS concentrations of 2500 - 3500 mg./L. the UNFED should be approximately 0.3 - 0.7 mg. DO/L./min.

To compare OUR on various sludges, the Specific Oxygen Uptake Rate (SOUR), a concentration independent value in mg. DO/hr./gm. can be determined:

- Calculate OUR in mg. DO/L./hr.: $OUR = (mg. DO/L./min.) \times (60 min./hr.) = mg. DO/L./hr.$
- Divide by MLVSS to obtain SOUR: $SOUR = (mg. DO/L./hr.) \times (1000 mg./gm.) / (mg./L. MLVSS)$

Example: OUR = 0.7 mg. DO/L./min.

MLVSS = 2500 mg./L.

$$SOUR = 0.7 \times 60 \times 1000 / 2500 = 16.8 mg. DO/hr./gm. MLVSS$$

DO/hr./gm. MLVSS 4.

Note: Optimum UNFED - OUR is about 12-20 mg. DO/hr./gm. MLVSS

Low UNFED <0.3 mg DO./L./min. or <0.5 mg. DO/hr./gm. MLVSS.

- Indicates starved, over-oxidized sludge (appropriate value for aerobic digester).
- Fast settling rates.
- Pin floc.
- Common to extended aeration systems - old sludge.
- Corrective Action - Increase wasting, decrease MLSS.

High UNFED >0.8 mg. DO/min. or >20 mg. DO/hr./gm. MLVSS.

- Indicates bulky, under-oxidized sludge (young).
- May go septic in clarifier.
- Poor settling and compactness.
- Corrective Action - Increase oxidation pressures.
 1. Contact or step-feed mode change may be required.
 2. Flocculation aid may be necessary to achieve settling and compactness.
 3. Monitor DO of return sludge and adjust to allow for maximum settleability w/o septicity.

Fed Rate

- FED follows availability and concentration of the feed. FED samples are more apt to change than UNFED. Measurement can provide time to anticipate likely changes and make corrections. FED will likely range from two to five (2 - 5) times the UNFED (0.6-3.5 mg. DO/L./min.).
- After feeding, OUR can be expected to increase relative to UNFED.
- Plant may impose limit on oxygenation capacity. Most systems should be able to handle FED of 2.0 - 2.5 mg./L./min. If not, or if FED higher, all or any below:
 1. improved mixing may be necessary.
 2. step-feeding to distribute load.
 3. more or modification of aeration.
- Toxic loads will depress FED relative to UNFED:
 1. treatment will suffer.
 2. coagulant or powdered activated carbon may help.
 3. if repeated, identify problem along collection system.
 4. if feed is suspect, check by using acceptable feed such as dextrose or sucrose to assess low activity.
 - if OUR increases. "bugs" are not assimilating food.
 - if OUR does not change, something in waste is toxic to "bugs".
 5. a small increase can be due to dilute feed, poor quality feed, sick sludge or unfavorable conditions (e.g. too long clarifier sludge detention time).

Load Index (LF - load factor).

- Ratio of FED to UNFED: (FED/UNFED).
- Indicates activity before and after feeding.
- Good sludge and acceptable feed increases LF >1.0 due to no depression in FED.
 1. LF <1.0 - inhibiting or toxic load.
 2. LF >1.0 but <2.0 - dilute or stabilized load.
 3. LF >2.0 but <5.0 - acceptable loading.
 4. LF >5.0 - possible O2 supply problems.

The oxygen uptake rate (OUR) test is a quantitative method of measuring the rate at which oxygen is being utilized by all the organisms in the process. It is expressed as mg/L of oxygen used per unit of time such as mg O₂/min or mg O₂/hr. However, this number by itself is not very useful since it only suggests the rate at which oxygen should be added to the process. However, it can be used effectively to determine when the treatment process has been completed by performing a stabilization test. It can also be used in the fed and unfed test to determine the effect of the influent on the activated sludge.

The fed and unfed tests use the simple OUR test on simulated sludges. A "fake" activated sludge is developed to simulate conditions at the influent end of the aeration tank. It is a fake test because of the difficulty of collecting a truly mixed sample at that point. Another problem is keeping the sample from changing between sampling and analysis. The validity of this test relies on the changeable behavior of microorganisms. Since the results of these tests must be related to each other, the unfed test uses the same organisms for a "fake" test to simulate the conditions at the effluent end of the aeration tank. The unfed test indicates how active the microorganisms are at rest (endogenous respiration conditions), while the fed test shows the microorganism activity after feeding.

The fed test mixes influent with the correct amount of return sludge. The unfed test mixes secondary clarifier effluent (unchlorinated) with the correct amount of return sludge. Then the normal OUR test is done on each. The correct volume of return sludge in ml for each test is calculated by:

$$mL \text{ of return sludge} = \frac{ATC}{RSC} \times 300mL$$

The 300 ml is the volume of a BOD bottle.

Fed/Unfed Ratios for Domestic Wastewater	
<1	⇒ Toxic effect.
1-2	⇒ Dilute load or material hard to stabilize.
2-5	⇒ Normal.
>5	⇒ Very high organic load for the TSU available.

Once the two tests have been completed, a ratio of fed over unfed is calculated. If the ratio is less than 1.0, a toxic effect is shown since the fed would be using oxygen slower than the unfed. A value of 1-2 is found with very dilute influents or with wastes that contain slowly-degradable materials. A ratio between 2-5 is usually normal for a domestic waste while a value above 5.0 indicates extremely high loading. Industrial wastes can greatly affect this ratio and their effects must be evaluated on a plant-by-plant basis.

The stabilization test is a fed test but done with a large container such as an aquarium or a barrel. It is done to determine the amount of time required for the microorganisms to stabilize the waste. The fed mixture is aerated and samples are taken every hour. OURs are run on each sample to show the reduction in OUR as the microorganisms stabilize the waste. When the OUR becomes constant, the waste treatment process has been completed. If the actual stabilization time is greater than the detention time provided in the aeration tank, then process changes must be made. The total amount of sludge in the system could be increased to provide more microbes to stabilize the waste faster. Or, the return rate could be reduced to increase the actual detention time. Or, another aeration tank would have to be put on line. Or, finally, the organic loading reduced.

Since the OUR measures the oxygen used in the process per unit time, it does not indicate how fast each microorganism is using oxygen. To determine that rate, the oxygen uptake rate must be divided by the concentration of microorganisms in the system. The resulting number is the specific oxygen uptake rate (SOUR) or respiration rate. It indicates the biological activity of an activated sludge mixed liquor or an aerobically digesting sludge. The procedure and analysis for activated sludge mixed liquor is presented here, but the procedure to be used for the Part 503 regulation for sludge digestion systems is given in Standard Methods (APHA, 1992) as Method 2710 B, Oxygen-Consumption Rate.

The SOUR for a mixed liquor is the amount of oxygen in mg/L utilized by one gram of volatile suspended solids in the activated sludge in one hour or mg O₂/g VSS/hour. It suggests how fast each microorganism is using oxygen or how fast it is metabolizing food.

The SOUR provides more information than the OUR test in that the actual rate of microorganism activity is determined. All the OUR test does is determine how fast the mass of microorganisms is using oxygen. However, the operator already knows this information based on the amount of DO in the aeration tank. If the DO is low, the microorganisms are using the O₂ faster than it is supplied. If the DO is high, the O₂ is being supplied at a rate faster than the microorganisms use it.

OUR Test**Equipment**

1. DO meter with BOD-bottle probe
2. 2-300 ml BOD bottles
3. Magnetic stirrer
4. Sample
5. Timer
6. Beaker with diameter slightly larger than BOD bottle

Procedure

1. Measure and record DO and temperature at the sample site.
2. Collect 3-4 liters of sample. The same sample can be used for suspended solids, settleability, centrifuge spins, microscopic exam, etc.
3. Pour some mixed liquor inside the beaker so that when the BOD bottle is placed in the beaker, the mixed liquor will come up to the neck of the bottle. This provides a more constant temperature in the BOD bottle during the test.
4. Thoroughly mix the sample, remove an aliquot and aerate it to get the DO above 5 mg/L. There are several methods that have been used to aerate the sample.
 - a. Fill a 300-ml BOD bottle with sample. Mix and aerate the sample by placing a PVC adapter between the filled bottle and an empty BOD bottle. Pour the sample back and forth to aerate.
 - b. Fill a one-liter container half full with mixed sample. Cap the container and gently shake. Fill the BOD bottle and begin.
5. Place the BOD bottle into the beaker. Be sure that the mixed liquor level in the beaker remains just below the neck of the BOD bottle.
6. Place the DO probe in the bottle, making sure not to trap any bubbles. Begin stirring the sample. Allow a short time for the temperature and the probe to stabilize.
7. Record the DO every minute for 10 minutes, or until the DO drop becomes consistent, or until the DO drops below 1 mg/L. Make sure that there is at least 1 mg/L difference between the start and finish of the test.

Note: If the OUR is extremely high, values may have to be read every 30 seconds and the initial DO may have to be increased to 7-8 mg/L. If that does not provide a satisfactory test, dilute the sample with BOD dilution water, reaerate the sample, and complete the test again. Calculate the appropriate dilution factor to obtain the OUR of the original sample.

OUR Calculations

1. Graph the "line of best fit" of the data. The line should be nearly straight. If there are data at the beginning and/or end of the test that are significantly different from the straight line, disregard those data. The straight line represents the actual microorganism activity.
2. Determine the slope of the line. Pick the points at the beginning and end of the straight-line portion. Divide the change in DO between those two points by the change in minutes between those two points.
3. $OUR = \Delta DO / \Delta \text{Time} = \text{mg O}_2/\text{L}/\text{min}$ if the time is in minutes

Multiply by 60 min/hr to get the results in $\text{mg O}_2/\text{L}/\text{hr}$

SOUR Calculations and Interpretation**Required information:**

OUR of the sample
MLSS or MLVSS of the same sample

Calculation

$$SOUR = \frac{OUR, \text{ mg/L/hr} \times 1,000 \text{ mg/gm}}{MLVSS, \text{ mg/L}}$$

Interpretation

Conventional activated sludge normal range - 12-20 $\text{mg O}_2/\text{hr}/\text{gm}$
Extended aeration normal range - 6-12 $\text{mg O}_2/\text{hr}/\text{gm}$

Values outside these ranges are not necessarily a problem since your plant may work well. However, they would normally mean that one should begin looking for potential problems.

Oxidation Reduction Potential Use in Wastewater Treatment

by

Brett Ward
Utility Operations Consultant
Municipal Technical Advisory Service
University of Tennessee

Oxidation Reduction Potential is an old test parameter that is seeing renewed popularity in wastewater applications. Readings give wastewater operators better insight into what wastewater microorganisms are actually doing in a particular situation or in a specific basin. This measurement parameter, which predates the dissolved oxygen meter, is useful in monitoring all types of biochemical reactions and is gaining popularity in monitoring wastewater processes which occur at dissolved oxygen levels below 1.0 mg/L. ORP readings also supplement D.O. readings by adding additional dimension of oxidative state to the positive D.O. readings. This ability to monitor and control anoxic and anaerobic respiration in a system can improve effluent quality, assist in odor control, and is key in effective biological nutrient removal. In the aeration basin ORP is proving to be effective in reducing operating cost while maintaining high effluent quality. ORP is also being used to monitor and control chlorination and dechlorination.

Oxidation Reduction Potential, also known as ORP, Redox, Redox potential, or electrical potential, is measured using the mV scale of a pH meter which is equipped with an ORP probe. The reading is a positive or negative number. The readings will tell an operator what the oxidizing state of the solution is at that time. Positive numbers indicate the presence of oxidizers or electron acceptors like oxygen and nitrate. Negative numbers indicate the presence of reduced compounds such as BOD and NH_3 , which need oxidizing in order for stabilization to take place. In the table (Gronoszy) shows different ORP readings, the electron acceptor and the type of conditions that exist at those mV levels. When aerobic or fully oxic conditions exist, oxygen is needed as the reduced compounds are oxidized. In these conditions BOD removal occurs, ammonia is oxidized into nitrate and polyphosphate development occurs. In anoxic conditions the facultative organisms use nitrate oxygen as an oxygen source and denitrification occurs. Polyphosphate breakdown occurs as the ORP declines below zero. At ORP readings of negative 50mV and below, anaerobic conditions exist. Here respiration creates odors, organic acids and methane.

Aerobic Processes

ORP levels of positive 50mV and above are in the oxic range, and aerobic respiration is taking place with oxygen being the final electron acceptor. Though a complex biochemical pathway, the net effect is that the bacteria oxidize the highly reduced or high energy organic matter in the waste stream transferring electrons to oxygen and producing CO_2 and H_2O and

1

energy that the bacteria use for maintenance, growth and reproduction. This process is basically the reverse of photosynthesis. Other aerobic processes are nitrification where ammonia is oxidized into nitrite and then nitrate. And polyphosphate is formed by the bacteria removing phosphorus from the water.

Anoxic Processes

At oxygen readings of 0.0 mg/L the bacteria must seek other sources of oxygen. The most easily available oxygen is that which is attached to nitrogen atoms forming nitrate (NO_3). When nitrate is available, the bacteria will denitrify the wastewater using the nitrate oxygen as an electron acceptor and releasing the nitrogen atom as nitrogen gas. If this occurs in the secondary clarifier, sludge clumping can be a problem and there can be solids washout; but if it occurs in the aeration basin at the operator's direction, the clarifier problems are avoided and the water has undergone biological nitrogen removal.

Anaerobic Processes

In order to biologically remove phosphate from wastewater there must be an anaerobic cycle where the polyphosphate bacteria use their stored polyphosphate energy and feed on the organic acids of the fermenting wastewater. This occurs at ORP readings of -50 to -200mV. In the wastewater collection system the formation of hydrogen sulfide occurs as the bacteria reduce sulfate in the water in their search for an electron acceptor as they oxidize fermentation products in the waste stream. As the electrical potential readings continue to decline, the acid forming and methane forming organisms begin to predominate the environment.

Instruments

Standard pH meters are used to measure ORP. The millivolt (mV) scale is used along with an ORP probe. Portable equipment can be purchased for less than \$800. The most common probe currently in use uses a silver/silver chloride reference electrode (Ag/AgCl). All readings included in this paper are or have been converted to Ag/AgCl values. Other reference electrodes are the Calome and Standard Hydrogen. When comparing ORP readings, you must know which reference electrode is being used. Standard Methods defines the electrical potentials (E) for the three electrodes as follows:

$$\text{ORP (E}_c\text{)} + 45\text{mV} = \text{ORP (E}_{\text{Ag}/\text{AgCl}}\text{)} + 210 = \text{ORP (E}_H\text{)}, (\text{Std. Methods})$$

There is no calibration of the meter when reading ORP, but the probe should be checked for accuracy using the available standards.

$$\begin{aligned} \text{Standards, milli Volts readings for the E}_{\text{Ag}/\text{AgCl}} \text{ electrode} \\ \text{Light's Solution} &= +475\text{mV} \\ \text{ZoBell's Solution} &= +228\text{mV} \end{aligned}$$

2

pH 4 and Quinhydrone = +263 mV
 pH 7 and Quinhydrone = +86 mV
 mV change from pH 4 to 7 should be 177mV \pm 20mV

If readings do not match the standards, the probe may need to be cleaned. Initial cleaning of a new probe should include liquid detergent, warm water and a soft brush followed by rinsing with DI water. Inorganic scale can be removed by soaking the probe for a few minutes in 5% Hydrochloric Acid. The platinum band may be polished with toothpaste. To remove oil and grease use acetone followed with detergent and rinsing (Broadley James). If the probe remains incorrect after cleaning, it should be discarded.

ORP Uses

ORP can be used to monitor and control a variety of wastewater processes and for spot checking of treatment plants and collection systems. Online controls of disinfection, aeration, sludge digestion and oxidant feed are all possible. Spot checking of wastewater systems using ORP can also be helpful in process control and troubleshooting.

Collection System

In the collection system odors begin to be produced at a reading of -50mV (Goronszy) though Mosey disputes this level saying the odors are not produced until -450mV. The difference may be the location of the measurement. Mosey's is a calculation based upon what the bacteria are doing in the slime layer, while Goronszy's appears to be a reading in the water above the slime. ORP measures can help operators pinpoint where in the collection system odor generation is most severe. They can also be used to monitor the dosage of various odor control oxidizers.

Aeration Basin

ORP is linearly related to the log of the dissolved oxygen. Goronszy reports that polyphosphate development begins and BOD removal begins at a reading of +50mV and ammonia removal at +100mV. Denitrification occurs in the anoxic zone or +50 to -50mV. Simultaneous nitrification and denitrification have been reported at +125mV by Muriyama. The theory is that outside the flock particle conditions are oxic but inside the flock particle there is a small anoxic zone where denitrification takes place.

When biological nutrient removal is being performed, ORP measurements give operators a definite on and off point for aeration control that incorporates not only the oxic spectrum where the D.O. meter can read, but also the anoxic and anaerobic spectrum where the D.O. meter cannot read. In situations where continuous ORP monitoring is performed on cycle aerated aeration basins a strip recorder will show definite inflection points on the plot of mV. As D.O. and ORP rise and fall, the rate of change in the mV reading has definite inflections at the points

where nitrification or denitrification has been completed. It is at these points that aeration is changed to suit the exact process needs. The importance of knowing when a certain process is completed is to prevent excess aeration and its added cost or insufficient aeration and its potential problems. Charpentier reports two activated sludge plants with 20% power savings using cycle aeration controlled by ORP.

Sludge Digestion

ORP can be used to control both aerobic and anaerobic sludge digesters. In the aerobic digester benefits of cycle aeration controlled by ORP or pH (Al-Ghusain) include alkalinity recovery, energy savings (to 43%, Yu) volatile solids reduction, fecal reduction and improved dewaterability (Peddie). As breakdown of solids occurs, expect longer cycles to be needed to reach desired ORP levels.

Molof reports that the optimum ORP for anaerobic digestion is - 400 to -500mV. He further recommends that probes be installed within the digester because of the slow response of the probes to these very low readings and the difficulty of making accurate readings in material withdrawn from the digester.

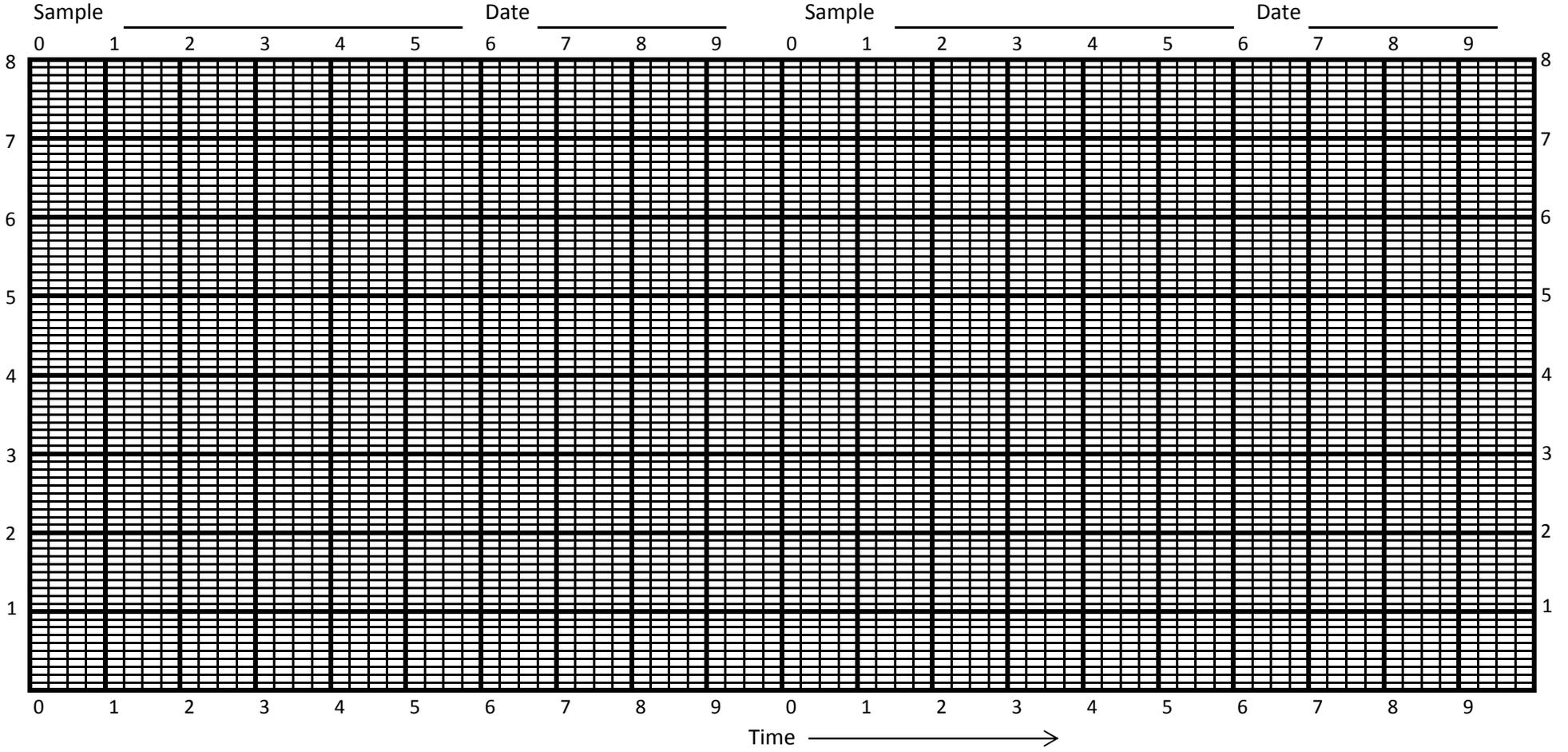
Disinfection

Several chlorinator manufacturers are using ORP instead of chlorine residual to control the feed of chlorine into contact chambers and the feed of dechlorinating materials. In a disinfection situation, ORP would be reading the combined oxidizing power of chlorine and oxygen in the water. Disinfection set points should be in the +500 to +550mV range (Spriggs). Kim reports a fecal coliform level of 2.2 MPN at an ORP of +520mV. The World Health Organization has set a drinking water standard at +650mV and +750mV is the European Pool and Spa standard. Dechlorination is made to a ORP reading of +200mV (Spriggs, Kim). An advantage of ORP control over chlorine residual is that as pH decreases and the killing power of chlorine ions increases, ORP also increases.

Summary

Oxidation Reduction Potential is another tool available to wastewater personnel. It's a way to better understand what the microorganisms are doing. And if we know what they are doing, we can predict what the results will be. For the operator or engineer who is working toward optimizing plant performance and economy, ORP values can be very helpful. By using online ORP measurements operators or computer control equipment can better manage the aeration process for nutrient removal. There can be significant power savings by cycling the aeration using ORP readings to determine the best off and on levels to prevent over aeration or under aeration. In the collection system, ORP readings can be used to locate odor producing thresholds in the system and to monitor oxidizer feed dosages used to prevent those odors. ORP can also be used to monitor both the aerobic and anaerobic digesters as well as the chlorination

Respiration Rate Worksheet/Graph



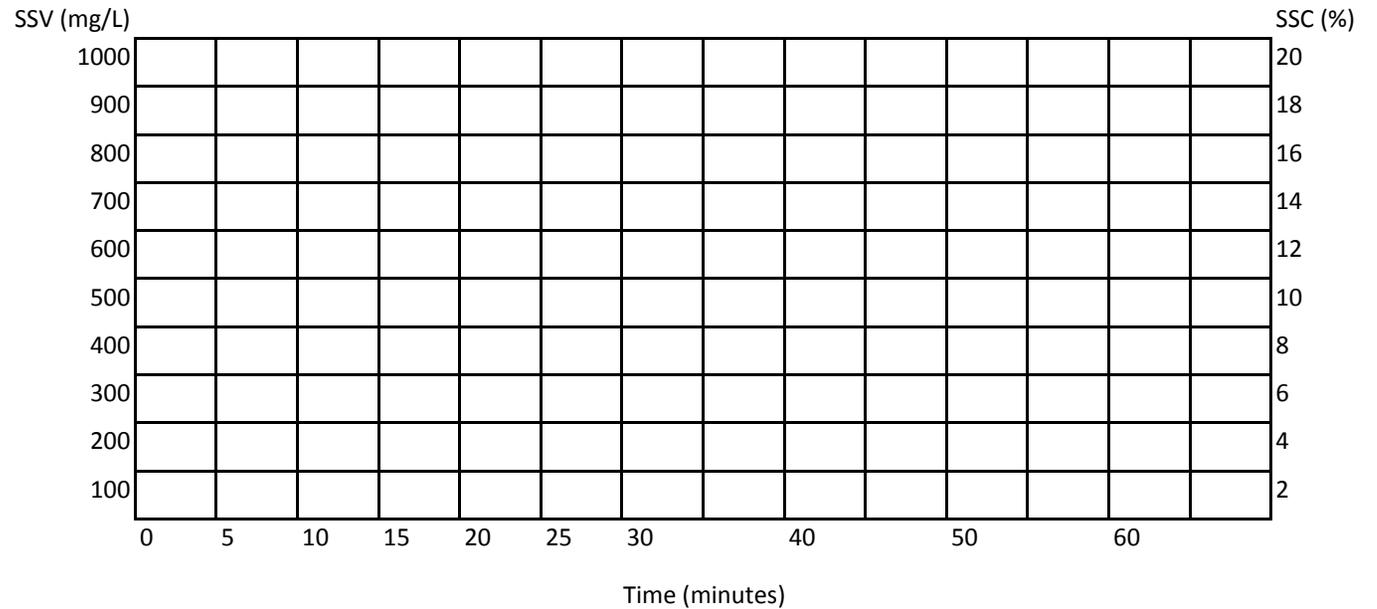
Slope Calculations	Slope Calculations
$\frac{\text{mg/L O}_2}{\text{min}} \times 60 = \text{mg O}_2/\text{L-hr (OUR)}$	$\frac{\text{mg/L O}_2}{\text{min}} \times 60 = \text{mg O}_2/\text{L-hr (OUR)}$
$\frac{\text{mg/L-hr OUR}}{\text{g/L MLVSS}} = \text{mg O}_2/\text{hr/g VSS}$	$\frac{\text{mg/L-hr OUR}}{\text{g/L MLVSS}} = \text{mg O}_2/\text{hr/g VSS}$

Settleability Bench Sheet

Date _____
 Time _____
 Analyst _____

Aeration Tank Concentration (ATC), % = _____

Time	SSV, mL/L	SSC, %
0		
5		
10		
15		
20		
25		
30		
40		
50		
60		



$$SSC = \frac{(1000)(ATC)}{SSV}$$

Observations:

Floc

- flocculant
- dispersed

Interface

- well defined
- ragged

Supernatant

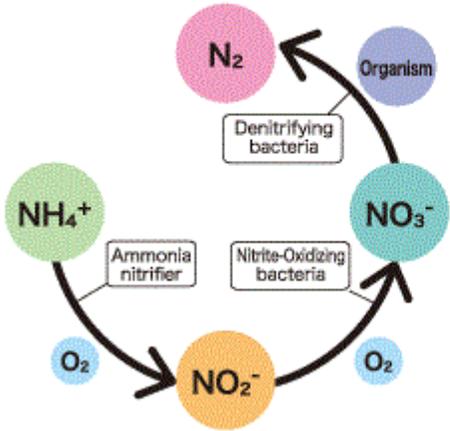
- clear
- turbid
- pin floc
- straggler floc

Rise Time, hrs: _____

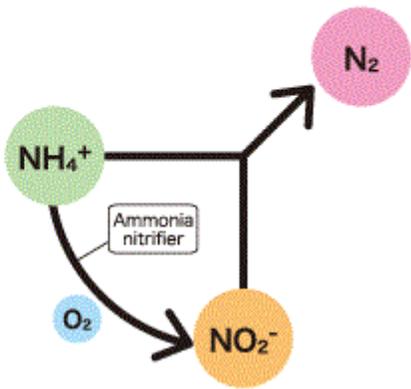
Comments: _____

Section 5 Nutrients

Reaction route of Conventional Nitrification/Denitrification



Denitrification using Anammox method



Nutrients

Nitrate-Nitrite, Ammonia
Total Kjeldahl Nitrogen,
Phosphorus



TDEC - Fleming Training Center 1

Nitrogen Group - N

- In water and wastewater the forms of nitrogen that are of greatest interest are:
 - Nitrate
 - Nitrite
 - Ammonia
 - Organic Nitrogen

} Inorganic Nitrogen } Total Nitrogen
 } Organic Nitrogen }

TDEC - Fleming Training Center 2

Phosphorus Group - P

- Phosphorus occurs in natural water and wastewater almost solely as phosphates
- Types of Phosphorus Analyses include:
 - Total Phosphorus
 - Ortho-Phosphorus
 - Dissolved Phosphorus

TDEC - Fleming Training Center 3

Containers, Preservation & Holding Times

Parameter	Container	Preservation	Max. Holding
Nitrate	P, G	Cool, ≤ 6° C	48 hours
Nitrite	P, G	Cool, ≤ 6° C	48 hours
Ortho-phosphate	P, G	Cool, ≤ 6° C	48 hours
Nitrate + Nitrite	P, G	Cool, ≤ 6° C, H ₂ SO ₄ to pH <2	28 days
Ammonia	P, G	Cool, ≤ 6° C, H ₂ SO ₄ to pH <2	28 days
Kjeldahl Nitrogen,	P, G	Cool, ≤ 6° C, H ₂ SO ₄ to pH <2	28 days
Phosphorus, Total	P, G	Cool, ≤ 6° C, H ₂ SO ₄ to pH <2	28 days

TDEC - Fleming Training Center 4

NO₃+NO₂ Methods for NPDES

Parameter	Methodology	Standard Methods
NO ₃ +NO ₂ -N	Ion Chromatography	4110 B – 2000 or C – 2000
	Automated Hydrazine	4500-NO ₃ H – 2000
	Cd-reduction, automated	4500-NO ₃ F – 2000
	Cd-reduction, manual	4500-NO ₃ E – 2000

TDEC - Fleming Training Center 5

Nitrite Methods for NPDES

Parameter	Methodology	Standard Methods
Nitrite (as N)	Ion Chromatography	4110 B – 2000 or C – 2000
	Spectrophotometric (Manual)	4500-NO ₂ B – 2000
	Automated, by pass Cd-reduction column	4500-NO ₃ F – 2000

TDEC - Fleming Training Center 6

Nitrate Methods for NPDES

Parameter	Methodology	Standard Methods
Nitrate (as N)	Ion Chromatography	4110 B – 2000 or C – 2000
	Ion Selective Electrode	4500-NO ₃ D – 2000
	Colorimetric (Brucine Sulfate)	No longer an approved method in Standard Methods
	Nitrate+Nitrite N Minus Nitrite N	Subtract value of Nitrite from value of Nitrate-Nitrite

TDEC - Fleming Training Center 7

Ammonia Methods for NPDES

Parameter	Methodology	Standard Methods
Ammonia, (as N)	Distillation or gas diffusion (pH>11) followed by:	4500-NH ₃ B – 97
	•Nesslerization	No longer an approved method in Standard Methods
	•Titration	4500-NH ₃ C – 97
	•Electrode	4500-NH ₃ D – 97 or E – 97
	•Automated phenate	4500-NH ₃ G – 97 or H – 97

TDEC - Fleming Training Center 8

TKN Methods for NPDES

Parameter	Methodology	Standard Methods
TKN (as N)	Digestion & Distillation followed by:	4500-N _{org} B – 97 or C – 97 & 4500-NH ₃ B – 97
	•Titration	4500-NH ₃ C – 97
	•Nesslerization	No longer an approved method in Standard Methods
	•Electrode	4500-NH ₃ D – 97 or 4500-NH ₃ E – 97

TDEC - Fleming Training Center 9

Total Phos. Methods for NPDES

Parameter	Methodology	Standard Methods
Phosphorus-Total	Persulfate digestion followed by:	4500-P B(5) – 99
	•Manual	4500-P E – 97
	•Automated ascorbic acid reduction	4500-P F – 99, G – 99 or H – 99

TDEC - Fleming Training Center 10

Ortho-Phos Methods for NPDES

Parameter	Methodology	Standard Methods
Ortho-Phosphate (P)	Ascorbic Acid Method:	
	•Automated	4500-P F – 99 or G – 99
	•Manual single reagent	4500-P E – 99

TDEC - Fleming Training Center 11

- ### Forms of Phosphates
- **Orthophosphate:** Produced by natural processes and are found in sewage
 - **Polyphosphate (or Metaphosphate):** Used for treating boiler waters and are in detergents.
 - In water they change into orthophosphate form
 - **Organic Bound Phosphate:** Result from breakdown of organic pesticides
- TDEC - Fleming Training Center 12

Environmental Impact

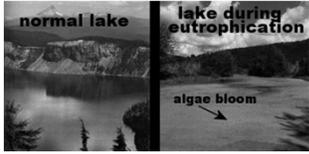
- Phosphate will stimulate the growth of aquatic plants
- High levels of phosphates entering waterways can stimulate algae and water plants to grow wildly which chokes up the waterways and uses up large amounts of dissolved oxygen



Algal growth fueled by nutrients running off agricultural fields and from urban areas) spreading into the Gulf of Mexico off the coast of Florida.

TDEC - Fleming Training Center 13

Environmental Impact



- Eutrophication or over-fertilization of receiving water from wastewater effluents
- Digestive problems can occur from extremely high levels of phosphate

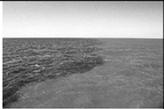
TDEC - Fleming Training Center 14

Eutrophication

- Eutrophication is an increase in chemical nutrients (compounds containing nitrogen or phosphorus) in an ecosystem, and may occur on land or in water.
- However, the term is often used to mean the resultant increase in the ecosystem's primary productivity (excessive plant growth and decay), and further effects including lack of oxygen and severe reductions in water quality, fish, and other animal populations.
- Once algae blooms, it will die off and as the algae decay bacteria will consume it and use up all the oxygen.

TDEC - Fleming Training Center 15

Eutrophication



- Gulf of Mexico
 - Currently the most notorious dead zone is a 8,543 mi² region in the Gulf of Mexico, where the Mississippi River dumps high-nutrient runoff from its vast drainage basin, which includes the heart of U.S. agribusiness, the Midwest.
 - The drainage of these nutrients are affecting important shrimp fishing grounds.
 - This is equivalent to a dead zone the size of New Jersey.

TDEC - Fleming Training Center 16

Eutrophication Video

Mississippi River Basin



TDEC - Fleming Training Center 17

Reversal of Dead Zones

- Dead zones are reversible.
- The Black Sea dead zone, previously the largest dead zone in the world, largely disappeared between 1991 and 2001 after fertilizers became too costly to use following the collapse of the Soviet Union and the demise of centrally planned economies in Eastern and Central Europe.
- Fishing has again become a major economic activity in the region

TDEC - Fleming Training Center 18

Most Common Procedures

- Orthophosphate is the amount of inorganic phosphorus in a sample and is measured by direct colorimetric procedures
- Total phosphorus is the amount of all phosphorus present in the sample regardless of form and is measured by the persulfate digestion procedure followed by the colorimetric analysis

TDEC - Fleming Training Center 19

Common Findings in Testing

Common Ranges	Influent	Effluent
Total Phosphorous	4 – 12 mg/L	2 – 10 mg/L
Orthophosphate	2 – 8 mg/L	1 – 6 mg/L

TDEC - Fleming Training Center 20

Acid Persulfate Digestion Method EPA Approved

- Gather sample
- Measure 50 ml into Erlenmeyer flask
- Add .5 grams of Potassium Persulfate and mix
- Add 2.0 ml of 5.25 Normality Sulfuric Acid Solution
- Place flask on hot plate and Boil gently for 30 minutes
- Cool sample to room temperature
- Add 2.0 ml of 5.0 Normality Sodium Hydroxide and mix
- Pour sample into 25 ml graduated cylinder and into clean sample bottle
- Proceed with reactive phosphorus test

- The digestive method is performed prior to testing for total phosphorus

TDEC - Fleming Training Center 21

Phosphorus PhosVer (Ascorbic Acid) Reactive Method

- Gather sample
- Fill a sample cell with one PhosVer 3 Phosphate powder pillow or use AccuVac Ampuls
- Swirl to mix reagent
- Two minute reaction time before testing
- Fill another sample cell with water from sample: This is your blank. Place in the cell holder of the analytical machine
- When timer beeps, the display will show mg/L P PV_press zero and wait for display to show 0.00 mg/L PO₄³⁻
- Place the sample into the machine and press read/enter (DR 4000 will do this automatically)

- If phosphate is present, the sample will turn BLUE in color.

TDEC - Fleming Training Center 22

Phosphorus SM4500-P B and E -1999

- DOC
- MDL
- LRB
- LFB
- LFM/LFMD
- ICAL/CCV
- Control Charts
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency



TDEC - Fleming Training Center 23

Phosphorus SM4500-P B and E -1999

- Demonstration of Capability (DOC)
 - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
 - Real people language: each operator running this test need to analyze 4 samples of a Phosphorus Standard at a concentration around 0.5 mg/L.
 - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
 - Recommend backup analyst do this once a year.

TDEC - Fleming Training Center 24

Phosphorus SM4500-P B and E - I 999

- MDL- Estimated Detection Level=0.01 mg/L
 - From SM 1030 C.
 - 0.01 mg/L * 5= 0.05 mg/L~ MDL
 - **Make a 0.05 mg/L standard**
 - **Analyze 7 portions over ≥ 3 days**
 - Calculate standard deviation (s)
 - $n1 \Sigma + n2 \Sigma + n3 \Sigma + \dots + n7 \Sigma + 2^{nd} \alpha xn = s$
 - $s * 3.14 = MDL$

TDEC - Fleming Training Center 25

Phosphorus SM4500-P B and E - I 999

- Method Blank – goes through digestion
 - Real people language: analyze distilled water as a sample by going through digestion and reagent addition before reading
 - Target value is less than reporting limit
 - Reporting limit will be equal to your Method Detection Limit (MDL)
 - Run on a 5% basis, one for every 20 samples
- Laboratory Fortified Blank – goes through digestion
 - Real people language: analyze a phosphorus standard at a concentration around 0.5 mg/L
 - Run on a 5% basis, one for every 20 samples

TDEC - Fleming Training Center 26

Phosphorus SM4500-P B and E - I 999

- Lab fortified matrix & duplicate (spike& spike dup)
 - 4020 B.2.g – When appropriate for the analyte, include at least one LFM/LFMD daily or with each batch of 20 or fewer samples
 - Add a known concentration of analyte (ideally from a second source) to a randomly selected routine sample without increasing its volume by more than 5%
 - Calculate percent recovery and relative percent difference, plot control charts and determine control limits for spikes at different concentrations
- Real people language – add a known amount of phosphorus to a sample and expect that amount to increase your sample concentration
 - Run on a 5% basis (1 for every 20 samples or once per month, whichever is more frequent)
 - Calculate RPD between spiked sample and spiked duplicate, target value should be close to the first value and have a small RPD (less than 20%).
 - Spike volume should be less than 1% of the volume.
 - Example: spike with 0.1 mL of 100 mg/L into 10 mL sample will equal a 1 mg/L increase in phosphorus concentration.

TDEC - Fleming Training Center 27

Phosphorus SM4500-P B and E - I 999

- Initial Calibration – does not go through digestion
 - Analyze 2-3 different standards within the curve
 - Run on a 5% basis, one for every 20 samples
- Calibration Verification – does not go through digestion
 - Analyze a mid-range phosphorous standard daily (day of)
 - Hach's method range is 0.2-2.50 mg/L, a 1 mg/L would work at your daily check standard

TDEC - Fleming Training Center 28

Phosphorus SM4500-P B and E - I 999

- **2014 Update** - Create and maintain control charts if you have 20-30 data points within 90 days.
 - If you do not meet the above criteria, follow QC Acceptance Criteria below.
 - Blanks < MDL
 - LFB $\pm 15\%$
 - ICV/CCV $\pm 10\%$
 - LFM/LFMD $\pm 20\%$
 - RPD < 20%
 - Reporting limit = MDL

TDEC - Fleming Training Center 29

Common Errors

- Sampling error
- Failure to analyze within holding
- Failure to use the correct method
- Failure to follow the method
- Failure to analyze the correct QC
- Calculation errors
- Standards or Reagents prepared incorrectly or expired

TDEC - Fleming Training Center 30

Procedural Concerns



- TKN & Total Phosphorus standards should be digested along with samples
- Phosphorus analyses require dedicated glassware

TDEC - Fleming Training Center 31

Procedural Concerns



- Ammonia distillation apparatus should be steamed out

TDEC - Fleming Training Center 32

Procedural Concerns (cont'd)

- Nitrate-Nitrite, cadmium column should be condition before use
- Verify efficiency of the cadmium column to reduce Nitrate to Nitrite

TDEC - Fleming Training Center 33

Bench Sheet Information

- Analysis & Method Number
- Analyst Initials and Date of Analysis
- Time of analysis (verify holding times)
- Sample ID
- Sample volumes used in prep/distillation
- Units
- Instrument used
- True Value of QC Samples

TDEC - Fleming Training Center 34

Phosphorus, Reactive (Orthophosphate)

DOC316.53.01119

USEPA¹ PhosVer 3 (Ascorbic Acid) Method²

Method 8048

0.02 to 2.50 mg/L PO₄³⁻

Powder Pillows or AccuVac® Ampuls

Scope and Application: For water, wastewater and seawater

¹ USEPA Accepted for reporting for wastewater analyses. Procedure is equivalent to USEPA and Standard Method 4500-P-E for wastewater.

² Adapted from *Standard Methods for the Examination of Water and Wastewater*.

! Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

Collect the following items:

Description	Quantity
Powder Pillow Test:	
PhosVer® 3 Phosphate Reagent powder pillow	1
Sample Cells, 1-inch, 10-mL	2
Stopper for 18 mm Tube (square sample cells only)	1
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
PhosVer® 3 Phosphate Reagent AccuVac® Ampul	1

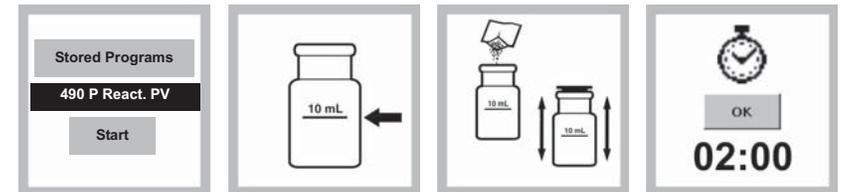
Phosphorus, Reactive (Orthophosphate)

Collect the following items: (continued)

Description	Quantity
Beaker, 50-mL	1
Sample Cell, 10-mL round	1
Stopper for 18-mm Tube (supplied with PhosVer AccuVacs)	1

See *Consumables and replacement items* for reorder information.

PhosVer 3 (Ascorbic Acid) method for powder pillows



- 1. Select the test.**
Insert an adapter if required (see *Instrument-specific information*).
- 2. Fill a sample cell with 10-mL of sample.**
- 3. Prepared Sample:**
Add the contents of one PhosVer 3 phosphate Powder Pillow to the cell. Immediately stopper and shake vigorously for 30 seconds.
- 4. Start the instrument timer.**
A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.



- 5. Blank Preparation:**
Fill a second sample cell with 10 mL of sample.
- 6.** When the timer expires, wipe the blank and insert it into the cell holder.
- 7. ZERO** the instrument.
The display will show: 0.00 mg/L PO₄³⁻
- 8.** Wipe the prepared sample and insert it into the cell holder.
READ the results in mg/L PO₄³⁻.

Phosphorus, Reactive (Orthophosphate)

PhosVer 3 (Ascorbic Acid) method for AccuVac® Ampuls



1. Select the test. Insert an adapter if required (see *Instrument-specific information*). Refer to the user manual for orientation.
2. **Blank Preparation:** Fill a sample cell with 10-mL of sample.
3. **Prepared Sample:** Fill a PhosVer 3 Phosphate AccuVac Ampul with sample. Keep the tip immersed while the Ampul fills completely.
4. Secure an Ampul cap over the tip of the Ampul. Shake the Ampul for approximately 30 seconds. Accuracy is unaffected by undissolved powder.



5. Start the instrument timer. A two-minute reaction period will begin. If the sample was digested using the Acid Persulfate digestion, a ten-minute reaction period is required.
6. When the timer expires, wipe the blank and insert it into the cell holder. **ZERO** the instrument. The display will show: 0.00 mg/L PO₄³⁻
7. Wipe the prepared sample and insert it into the cell holder. **READ** the results in mg/L PO₄³⁻.

Phosphorus, Reactive (Orthophosphate)

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level.
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Hydrogen Sulfide	Interferes at any level
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. pH 2–10 is recommended.
Silica	Greater than 50 mg/L
Silicate	Greater than 10 mg/L
Turbidity or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment ¹ Powder Pillow to 25 mL of sample. Mix well. Use this solution to zero the instrument.
Zinc	Greater than 80 mg/L

¹ See *Optional reagents and apparatus*.

Sample collection, preservation and storage

- Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.
- For best results, analyze samples immediately.
- If prompt analysis is not possible, preserve samples by filtering immediately and storing at 4 °C (39 °F) for up to 48 hours.
- Return the sample to room temperature before analysis.

Accuracy check

Standard additions method (sample spike)

Required for accuracy check:

- Phosphate 10-mL Ampule Standard, 50-mg/L PO₄³⁻
- Ampule breaker
- TenSette Pipet

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.

* See *Optional reagents and apparatus*.

Phosphorus, Reactive (Orthophosphate)

- Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADDITIONS
DR 2800	OPTIONS>MORE>STANDARD ADDITIONS
DR 2700	OPTIONS>MORE>STANDARD ADDITIONS
DR/2500	OPTIONS>STANDARD ADDITIONS
DR/2400	OPTIONS>STANDARD ADDITIONS

- Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
- Open the standard solution ampule.
- Prepare a 0.1-mL sample spike by adding 0.1 mL of standard to the unspiked sample. Press the timer icon. After the timer expires, read the result.
- Prepare a 0.2-mL sample spike by adding 0.1 mL of standard to the 0.1-mL sample spike. Press the timer icon. After the timer expires, read the result.
- Prepare a 0.3-mL sample spike by adding 0.1 mL of standard to the 0.2-mL sample spike. Press the timer icon. After the timer expires, read the result. Each addition should reflect approximately 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)

Required for accuracy check:

- Mixing cylinders (3)
- Fill three mixing cylinders each with 50-mL of sample and spike with 0.2 mL, 0.4 mL and 0.6 mL of standard.
 - Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
 - Analyze each standard addition sample as described in the *PhosVer 3 (Ascorbic Acid) method for AccuVac® Ampuls*.
 - Accept each standard additions reading. Each addition should reflect approximately 100% recovery.

Standard solution method

Note: Refer to the instrument user manual for specific software navigation instructions.

Required for accuracy check:

- Phosphate standard solution, 50 mg/L
 - Deionized water
 - 100-mL Class A volumetric flask
 - Class A volumetric pipet
 - TenSette Pipet
- Prepare a 2.00 mg/L phosphate standard solution as follows:
 - Pipet 4.00 mL of Phosphate Standard, 50-mg/L, into a 100-mL volumetric flask.

Phosphorus, Reactive (Orthophosphate)

- Dilute to volume with demineralized water. Mix well. Prepare this solution daily.

Note: Alternately, use one of the mixed parameter standards listed in Recommended standards. These contain 2.0 mg/L phosphate.

- Use this solution in place of the sample. Follow the *PhosVer 3 (Ascorbic Acid) method for powder pillows* test procedure.
- To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADJUST
DR 2800	OPTIONS>MORE>STANDARD ADJUST
DR 2700	OPTIONS>MORE>STANDARD ADJUST
DR/2500	OPTIONS>STANDARD ADJUST
DR/2400	OPTIONS>STANDARD ADJUST

- Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
490	DR 5000	2.00 mg/L PO ₄ ³⁻	1.98–2.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR 2800	2.00 mg/L PO ₄ ³⁻	1.98–2.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR 2700	2.00 mg/L PO ₄ ³⁻	1.98–2.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR/2500	1.00 mg/L PO ₄ ³⁻	0.97–1.03 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR/2400	1.00 mg/L PO ₄ ³⁻	0.97–1.03 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
492	DR 5000	2.00 mg/L PO ₄ ³⁻	1.98–2.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR 2800	2.00 mg/L PO ₄ ³⁻	1.98–2.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR 2700	2.00 mg/L PO ₄ ³⁻	1.98–2.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR/2500	1.00 mg/L PO ₄ ³⁻	0.98–1.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻
	DR/2400	1.00 mg/L PO ₄ ³⁻	0.98–1.02 mg/L PO ₄ ³⁻	0.02 mg/L PO ₄ ³⁻

Summary of method

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm

Phosphorus, Reactive (Orthophosphate)

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
PhosVer® 3 Phosphate Reagent Powder Pillows, 10-mL OR	1	100/pkg	2106069
PhosVer® 3 Phosphate Reagent AccuVac® Ampuls	1	25/pkg	2508025

Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
Stopper for 18 mm Tube	1	6/pkg	173106

Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H
Stopper for 18 mm Tube	1	6/pkg	173106

Recommended standards

Description	Unit	Catalog number
Phosphate Standard Solution, 10-mL Voluette® Ampul, 50-mg/L as PO ₄	16/pkg	17110
Phosphate Standard Solution, 50-mg/L as PO ₄	500 mL	17149
Phosphate Standard Solution, 1-mg/L as PO ₄	500 mL	256949
Standard, Drinking Water, Mixed Parameter, Inorganic: F, NO ₃ , PO ₄ , SO ₄	500 mL	2833049
Wastewater Effluent Standard, for mixed parameters: NH ₃ -N, NO ₃ -N, PO ₄ , COD, SO ₄ , TOC	500 mL	2833249
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Hydrochloric Acid Solution, 6.0N, 1:1	500 mL	88449
Mixing Cylinder 50 mL	each	189641
Phosphate Treatment Powder Pillow	100/pkg	1450199
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001 ¹	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001 ¹	1000/pkg	2185628
Pipet, TenSette, Pipet, 1.0 - 10.0 mL	each	1970010
Pipet Tips, for TenSette Pipet 1970010 ¹	50/pkg	2199796
Pipet Tips, for TenSette Pipet 1970010 ¹	250/pkg	2199725
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076

Phosphorus, Reactive (Orthophosphate)
Page 7 of 8

Phosphorus, Reactive (Orthophosphate)

Optional reagents and apparatus

Description	Unit	Catalog number
pH Paper, 0–14 pH range	100/pkg	2601300
AccuVac snapper	each	2405200
AccuVac ampule blanks	25/pkg	2677925
Flask, volumetric, 100 mL	each	1457442
Pipet, volumetric, Class A, 4 mL	each	1451504
AccuVac ampule drainer	each	4103600

¹ Other sizes are available

Optional standards

Description	Unit	Catalog number
Voluette Ampule breaker 10 mL	each	2196800
Phosphate, 10 mg/L	946 mL	1420416
Phosphate, 15 mg/L	100 mL	1424342
Phosphate; 100 mg/L	100 mL	1436832
Phosphate; 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate; 500 mg/L	100 mL	1424232

 FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

© Hach Company, 2007. All rights reserved. Printed in the U.S.A.

Updated February 2008, Edition 5

Phosphorus, Total, Digestion

DOC316.53.01112

USEPA¹ Acid Persulfate Digestion Method²

Method 8190

Scope and Application: For water, wastewater and seawater

¹ USEPA Accepted for wastewater analyses when used with the ascorbic acid (PhosVer 3) method.

² Adapted from *Standard Methods for the Examination of Water and Wastewater* 4500-P B & E

Test preparation

Before starting the test:

Rinse all glassware with 1:1 hydrochloric acid. Rinse again with deionized water.

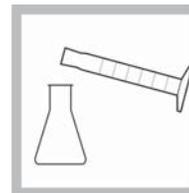
Collect the following items:

Description	Quantity
Potassium Persulfate Powder Pillows	1
Sodium Hydroxide Solution, 5.0 N	2 mL
Sulfuric Acid Solution, 5.25 N	2 mL
Water, deionized	varies
Cylinder, graduated, 25-mL	1
Flask, Erlenmeyer, 125-mL	1
Hot Plate	1

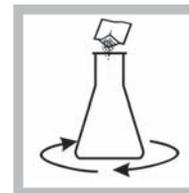
See *Consumables and replacement items* for reorder information.

Phosphorus, Total, Digestion

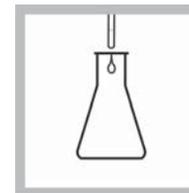
Acid Persulfate Digestion



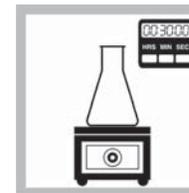
1. Use a graduated cylinder to measure 25 mL of sample. Pour the sample into a 125-mL Erlenmeyer flask.



2. Add the contents of one Potassium Persulfate Powder Pillow. Swirl to mix.



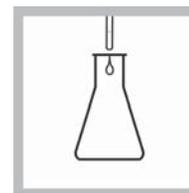
3. Use a 1-mL calibrated dropper to add 2.0 mL of 5.25 N Sulfuric Acid Solution to the flask.



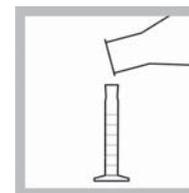
4. Place the flask on a hot plate. Boil gently for 30 minutes. Do not boil dry. Concentrate the sample to less than 20 mL for best recovery. After concentration, maintain the volume near 20 mL by adding small amounts of deionized water. Do not exceed 20 mL.



5. Cool the sample to room temperature.



6. Use a 1-mL calibrated dropper to add 2.0 mL of 5.0 N Sodium Hydroxide Solution to the flask. Swirl to mix.



7. Pour the sample into a 25-mL graduated cylinder. Adjust the volume to 25 mL with deionized water rinsings from the flask.

480 P React. Mo
482 P React. Mo. AV
485 P React. Amino
490 P React. PV
492 P React. PV AV
535 P React. PV TNT
540 P React. HT TNT

8. Proceed with a reactive phosphorus test of the expected total phosphorus concentration range.

Extend the color development time to 10 minutes for the PhosVer 3 (ascorbic acid) method.

Phosphorus, Total, Digestion

Interferences

Table 1 Interfering substances

Interfering substance	Interference level
Alkaline or highly buffered samples	It may be necessary to add additional acid in step 3 to drop the pH of the solution below 1.
Turbidity	Use 50 mL of sample and double the reagent quantities. Use a portion of the reacted sample to zero the instrument in the reactive phosphorus procedure. This compensates for any color or turbidity destroyed by this procedure.

Sample collection, preservation and storage

- Analyze the samples immediately for the most reliable results.
- If prompt analysis is not possible, samples may be preserved up to 28 days.
- To preserve samples, adjust the pH to 2 or less with Concentrated Sulfuric Acid[†] (about 2 mL per liter). Store at 4 °C.
- Warm the sample to room temperature and neutralize with 5.0 N Sodium Hydroxide before analysis.
- Correct test results for volume additions.

Summary of method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphate by heating with acid and persulfate. Organically bound phosphates are thus determined indirectly by subtracting the result of an acid hydrolyzable phosphorus test from the total phosphorus result.

This procedure must be followed by one of the reactive phosphorus (orthophosphate) analysis methods for determining the phosphorus content of the sample. If the ascorbic acid (PhosVer 3) method is used to measure the reactive phosphorus, this method is USEPA accepted for NPDES reporting.

The following reagents and apparatus are required in addition to those required for the active phosphorus test.

* See *Optional reagents*.

Phosphorus, Total, Digestion

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Potassium Persulfate Powder Pillows	1	100/pkg	245199
Sodium Hydroxide Solution, 5.0 N	2 mL	100 mL MDB	245032
Sulfuric Acid Solution, 5.25 N	2 mL	100 mL MDB	244932
Water, deionized	varies	4 L	27256

Required apparatus

Description	Quantity/Test	Unit	Catalog number
Cylinder, graduated, 25-mL	1	each	50840
Flask, Erlenmeyer, 125-mL	1	each	50543
Hot Plate, 7" x 7" digital, 240 VAC	1	each	2881500
Hot Plate, 7" x 7" digital, 240 VAC	1	each	2881502

Optional reagents

Description	Unit	Catalog number
Sodium, Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
pH paper, 0–14 pH range	100/pkg	2601300
Thermometer, Non-Mercury, –10 to 225°C	each	2635700
Sampling bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
Hydrochloric Acid, 6.0 N	500 mL	88449



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Phosphorus, Total

DOC316.53.01121

USEPA¹
PhosVer® 3 with Acid Persulfate Digestion Method

Method 8190

0.06 to 3.50 mg/L PO₄³⁻ or
0.02 to 1.10 mg/L P

Test 'N Tube™ Vials

Scope and Application: For water, wastewater and seawater

¹ USEPA Accepted for reporting wastewater analyses (Standard Methods 4500 P-E).

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Light shield	Adapter
DR 5000	—	—
DR 2800	LZV646	—
DR 2700	LZV646	—
DR/2500	—	—
DR/2400	—	5945700

Before starting the test:

DR 2800 and DR 2700 only: Install the light shield in Cell Compartment #2 before performing this test.

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water in place of the sample. Subtract the reagent blank value from the final results or perform a reagent blank adjust.

The test range for total phosphate is limited to 0.06 to 3.5 mg/L PO₄³⁻. Values greater than 3.5 mg/L may be used to estimate dilution ratios, but should NOT be used for reporting purposes. If the value is greater than 3.5 mg/L, dilute the sample and repeat the digestion and the colorimetric test.

Final samples will contain molybdenum. In addition, final samples will have a pH less than 2 and are considered corrosive (D002) by the Federal RCRA. Refer to the current MSDS for safe handling and disposal instructions.

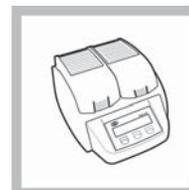
Phosphorus, Total

Collect the following items:

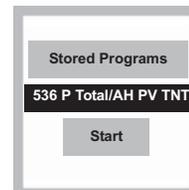
Description	Quantity
Total Phosphorus Test 'N Tube™ Reagent Set	1
Deionized water	varies
DRB200 Reactor	1
Funnel, micro	1
Light Shield or Adapter (see <i>Instrument-specific information</i>)	1
Pipet, TenSette®, 1 to 10 mL, plus tips	1
Test Tube Rack	1

See *Consumables and replacement items* for reorder information.

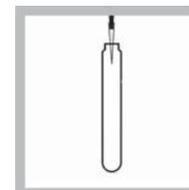
PhosVer 3, acid persulfate digestion



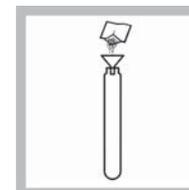
1. Turn on the DRB200 Reactor. Preheat to 150 °C.



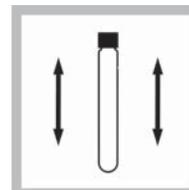
2. Select the test. Insert an adapter if required (see *Instrument-specific information*).



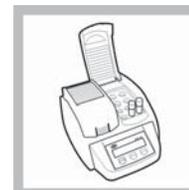
3. Use a TenSette® Pipet to add 5.0 mL of sample to a Total Phosphorus Test Vial.



4. Use a funnel to add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.



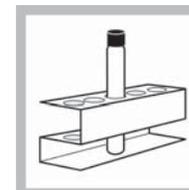
5. Cap tightly and shake to dissolve.



6. Insert the vial into the DRB200. Close the protective cover.



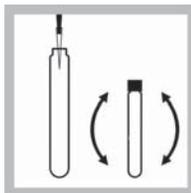
7. Set the instrument timer to 30 minutes and start. A 30-minute heating period will begin.



8. When the timer expires, carefully remove the hot vial from the reactor. Insert it in a test tube rack and cool to room temperature.

Phosphorus, Total

PhosVer 3, acid persulfate digestion (continued)



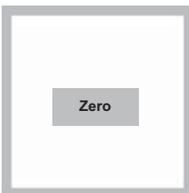
9. Use a TenSette Pipet to add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial. Cap and mix.



10. Wipe the outside of the vial with a damp cloth followed by a dry one.



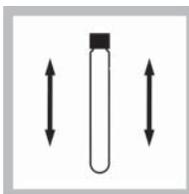
11. Insert the vial into the 16 mm cell holder.



12. ZERO the instrument. The display will show: 0.00 mg/L PO₄³⁻



13. Use a funnel to add the contents of one PhosVer 3 Powder Pillow to the vial.



14. Immediately cap tightly and shake to mix for 20–30 seconds. The powder will not dissolve completely.



15. Start the instrument timer. A two-minute reaction period will begin. Read the sample within 2–8 minutes after the timer expires.



16. After the timer expires, wipe the outside of the vial with a wet towel, then a dry one. Insert the prepared sample vial into the 16 mm cell holder. READ the results in mg/L PO₄³⁻.

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Aluminum	Greater than 200 mg/L
Arsenate	Interferes at any level
Chromium	Greater than 100 mg/L
Copper	Greater than 10 mg/L
Iron	Greater than 100 mg/L
Nickel	Greater than 300 mg/L
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Silica	Greater than 50 mg/L

Phosphorus, Total

Table 2 Interfering substances (continued)

Interfering substance	Interference level
Silicate	Greater than 10 mg/L
Sulfide	Greater than 90 mg/L
Turbidity or color	May cause inconsistent results because the acid in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Zinc	Greater than 80 mg/L

Sample collection, preservation and storage

- Collect samples in plastic or glass bottles that have been acid washed with 1:1 Hydrochloric Acid Solution* and rinsed with deionized water.
- Do not use commercial detergents containing phosphate for cleaning glassware used in this test.
- Analyze the samples immediately for the most reliable results.
- If prompt analysis is not possible, samples may be preserved up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric Acid* (about 2 mL per liter) and storing at 4 °C.
- Warm stored samples to room temperature and neutralize with 5.0 N Sodium Hydroxide* before analysis.
- Correct test results for volume additions.

Accuracy check

Standard additions method (sample spike)

Required for accuracy check:

- Phosphate 10-mL Ampule Standard, 50-mg/L as PO₄³⁻
- Ampule breaker
- TenSette Pipet
- Mixing cylinders, (3)

- Clean glassware with 1:1 Hydrochloric Acid Standard Solution. Rinse again with deionized water. Do not use phosphate detergents to clean glassware.
- After reading test results, leave the sample cell (unspiked sample) in the instrument.
- Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADDITIONS
DR 2800	OPTIONS>MORE>STANDARD ADDITIONS
DR 2700	OPTIONS>MORE>STANDARD ADDITIONS
DR/2500	OPTIONS>STANDARD ADDITIONS
DR/2400	OPTIONS>STANDARD ADDITIONS

- Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.

* See *Optional reagents and apparatus*.

Phosphorus, Total

- Open the standard solution ampule.
- Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 25-mL portions of fresh sample.
- Use a 5-mL aliquot of the spiked sample in place of the sample. Follow the *PhosVer 3, acid persulfate digestion* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
- Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: Refer to the instrument user manual for specific software navigation instructions.

Required for accuracy check:

- Phosphate standard solution, 3.0-mg/L
- Use the 3.0 mg/L phosphate standard solution in place of the sample. Follow the *PhosVer 3, acid persulfate digestion* test procedure.
 - To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADJUST
DR 2800	OPTIONS>MORE>STANDARD ADJUST
DR 2700	OPTIONS>MORE>STANDARD ADJUST
DR/2500	OPTIONS>STANDARD ADJUST
DR/2400	OPTIONS>STANDARD ADJUST

- Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity— Δ Concentration per 0.010 Δ Abs
536	DR 5000	3.00 mg/L PO ₄ ³⁻	2.93–3.07 mg/L PO ₄ ³⁻	0.06 mg/L PO ₄ ³⁻
	DR 2800	3.00 mg/L PO ₄ ³⁻	2.93–3.07 mg/L PO ₄ ³⁻	0.06 mg/L PO ₄ ³⁻
	DR 2700	3.00 mg/L PO ₄ ³⁻	2.93–3.07 mg/L PO ₄ ³⁻	0.06 mg/L PO ₄ ³⁻
	DR/2500	3.00 mg/L PO ₄ ³⁻	2.90–3.10 mg/L PO ₄ ³⁻	0.06 mg/L PO ₄ ³⁻
	DR/2400	3.00 mg/L PO ₄ ³⁻	2.90–3.10 mg/L PO ₄ ³⁻	0.06 mg/L PO ₄ ³⁻

Summary of method

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) must be converted to reactive orthophosphate before analysis. Pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are converted to orthophosphates by heating with acid and persulfate.

Phosphorus, Total

Orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color. Test results are measured at 880 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Total Phosphorus Test 'N Tube™ Reagent Set, 50 tests, includes:	—	—	2742645
PhosVer® 3 Phosphate Reagent Powder Pillows	1	50/pkg	2106046
Potassium Persulfate Powder Pillows	1	50/pkg	2084766
Sodium Hydroxide Solution, 1.54 N	2 mL	100 mL	2743042
Total and Acid Hydrolyzable Test Vials ¹	1	50/pkg	—
Water, deionized	varies	100 mL	27242

¹ Not sold separately

Required apparatus

Description	Quantity/Test	Unit	Catalog number
DRB200 Reactor, 110 V, 15 x 16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15 x 16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Pipet, TenSette®, 1.0 to 10 mL	1	each	1970010
Pipet Tips for TenSette Pipet 19700-10	1	250/pkg	2199725
Test Tube Rack	1	each	1864100

Recommended standards

Description	Unit	Catalog number
Drinking Water Standard, Mixed Parameter, Inorganic for F-, NO ₃ , PO ₄ , SO ₄	500 mL	2833049
Phosphate Standard Solution, 10-mL Voluette® Ampule, 50-mg/L as PO ₄ ³⁻	16/pkg	17110
Phosphate Standard Solution, 1-mg/L as PO ₄ ³⁻	500 mL	256949
Phosphate Standard Solution, 3 mg/L as PO ₄ ³⁻	946 mL	2059716
Wastewater Standard, Effluent Inorganics, for NH ₃ -N, NO ₃ -N, PO ₄ , COD, SO ₄ , TOC	500 mL	2833249
Voluette Ampule breaker 10 mL	each	2196800

Optional reagents and apparatus

Description	Unit	Catalog number
Cylinder, mixing	25 mL	189640
Pipet, volumetric, Class A, 2.00 mL	1	each
Hydrochloric Acid Solution, 6.0 N, 1:1	500 mL	88449
Sodium Hydroxide, 5.0 N	1000 mL	245053
Sulfuric Acid, concentrated	500 mL	97949
Pipet, TenSette® Pipet, 0.1–1.0 mL	each	1970001

Phosphorus, Total**Optional reagents and apparatus (continued)**

Description	Unit	Catalog number
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
pH Paper, 0–14 pH range	100/pkg	2601300
Deionized Water	4 L	27256
Thermometer, Non-Mercury, -10 to 225 °C	each	2635700
Finger cots	2/pkg	1464702

Optional standards

Description	Unit	Catalog number
Phosphate, 10 mg/L	946 mL	1420416
Phosphate, 15 mg/L	100 mL	1424342
Phosphate, 100 mg/L	100 mL	1436832
Phosphate, 500 mg/L, 10 mL Voluette Ampules	16/pkg	1424210
Phosphate, 500 mg/L	100 mL	1424232



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Nitrogen, Total

DOC316.53.001086

Persulfate Digestion Method

Method 10071

LR (0.5 to 25.0 mg/L N)

Test 'N Tube™ Vials

Scope and Application: For water and wastewater

! Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Light shield	Adapter
DR 5000	—	—
DR 2800	LZV646	—
DR 2700	LZV646	—
DR/2500	—	—
DR/2400	—	5945700

Before starting the test:

DR 2800 and DR 2700 only: Install the light shield in Cell Compartment #2 before performing this test.

Digestion is required for determining total nitrogen.

This test is technique-sensitive. Invert the vials as described here to avoid low results: Hold the vial in a vertical position with the cap pointing up. Turn the vial upside-down. Wait for all of the solution to flow down to the cap. Pause. Return the vial to an upright position. Wait for all the solution to flow to the bottom of the vial. This process equals one inversion.

If the test overranges, repeat the digestion and measurement with diluted sample. The digestion must be repeated for accurate results.

Use the deionized water provided in the reagent set or Organic-free Water to prepare the standards and perform the procedure.

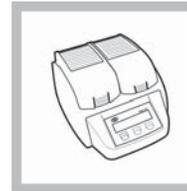
Collect the following items:

Description	Quantity
Test 'N Tube™ LR Total Nitrogen Reagent Set	1
DRB200 Reactor	1
Funnel, micro	1
Light Shield or adapter (see <i>Instrument-specific information</i>)	1
Pipet, TenSette®, 1.0 to 10.0 mL plus tips	1
Test Tube Cooling Rack	1-3
Finger Cots	2

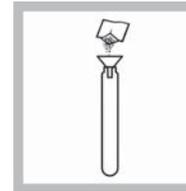
See *Consumables and replacement items* for reorder information.

Nitrogen, Total

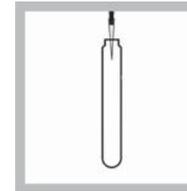
Persulfate digestion method



1. Turn on the DRB200 Reactor and heat to 105 °C.

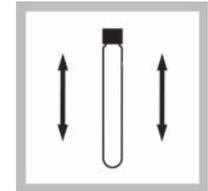


2. Using a funnel, add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two Total Nitrogen Hydroxide Digestion Reagent vials. Wipe off any reagent that may get on the lid or the tube threads.
Note: One reagent blank is sufficient for each set of samples.



3. **Prepared Sample:** Add 2 mL of sample to one vial.
Blank Preparation: Add 2 mL of the deionized water included in the kit to a second vial.

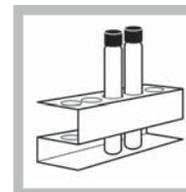
Use only water that is free of all nitrogen-containing species as a substitute for the provided deionized water.



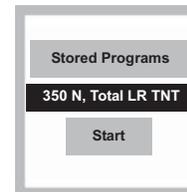
4. Cap both vials. Shake vigorously for at least 30 seconds to mix. The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.



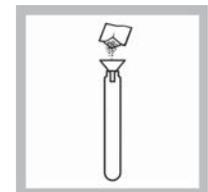
5. Insert the vials in the reactor and close the lid.
Heat for exactly 30 minutes.



6. Using finger cots, **immediately** remove the hot vials from the reactor. Cool the vials to room temperature.



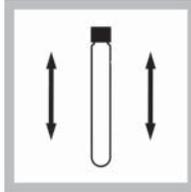
7. Select the test. Insert an adapter if required (see *Instrument-specific information*).



8. Remove the caps from the digested vials and add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.

Nitrogen, Total

Persulfate digestion method (continued)



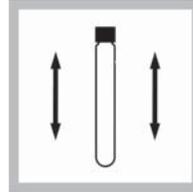
9. Cap the tubes and shake for 15 seconds.



10. Start the instrument timer.
A three-minute reaction period will begin.



11. After the timer expires, remove the caps from the vials and add one TN Reagent B Powder Pillow to each vial.



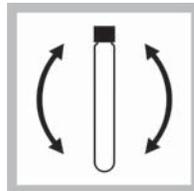
12. Cap the tubes and shake for 15 seconds. The reagent will not completely dissolve. This will not affect accuracy. The solution will begin to turn yellow.



13. Start the instrument timer.
A two-minute reaction period will begin.



14. After the timer expires, remove the caps from two TN Reagent C vials and add 2 mL of digested, treated sample to one vial. Add 2 mL of digested, treated reagent blank to the second TN Reagent C vial.



15. Cap the vials and invert ten times to mix. Use slow, deliberate inversions for complete recovery.
The tubes will be warm to the touch.



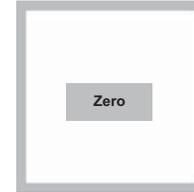
16. Start the instrument timer.
A five-minute reaction period will begin.
The yellow color will intensify.

Nitrogen, Total

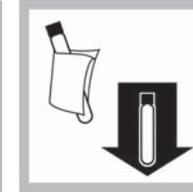
Persulfate digestion method (continued)



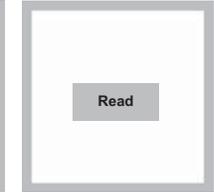
17. Wipe the reagent blank and insert it into the 16-mm round cell holder.



18. ZERO the instrument.
The display will show:
0.0 mg/L N



19. Wipe the reagent vial and insert it into the 16-mm round cell holder.
Note: Multiple samples may be read after zeroing on one reagent blank.



20. READ the results in mg/L N.

Blanks for colorimetric measurement

The reagent blank may be used up to seven days for measurements using the same lots of reagents. Store it in the dark at room temperature (18–25 °C). If a small amount of white floc appears within a week, discard the reagent blank and prepare a new one.

Interferences

The *Non-interfering substances* table shows substances that have been tested and found not to interfere up to the indicated levels (in mg/L). Interfering substances that resulted in a concentration change of ±10% appear in the *Interfering substances* table.

Table 2 Non-interfering substances

Interfering substance	Interference level
Barium	2.6 mg/L
Calcium	300 mg/L
Chromium (3+)	0.5 mg/L
Iron	2 mg/L
Lead	6.6 µg/L
Magnesium	500 mg/L
Organic Carbon	150 mg/L
pH	13 pH units
Phosphorus	100 mg/L
Silica	150 mg/L
Silver	0.9 mg/L
Tin	1.5 mg/L

Table 3 Interfering substances

Interfering substance	Interference level
Bromide	> 60 mg/L; positive interference

Nitrogen, Total

Table 3 Interfering substances (continued)

Interfering substance	Interference level
Chloride	> 1000 mg/L; positive interference

This test performed with standard nitrogen solutions prepared from the following compounds obtained 95% recovery:

- Ammonium chloride
- Ammonium sulfate
- Ammonium acetate
- Urea
- Glycine

Ammonium chloride or nicotinic-PTSA spikes in domestic influent, effluent and the ASTM standard specification for substitute wastewater (D 5905-96) also resulted in $\geq 95\%$ recovery.

The large amounts of nitrogen-free organic compounds in some samples may decrease digestion efficiency by consuming some of the persulfate reagent. Samples known to contain high levels of organics should be diluted and re-run to verify digestion efficiency.

Sample collection, storage and preservation

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
- Preserve the sample by reducing the pH to 2 or less with concentrated (at least 2 mL/L) Sulfuric Acid.
- Store samples at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days.
- Warm stored samples to room temperature and neutralize with 5 N Sodium Hydroxide before analysis.
- Correct the test result for volume additions.

Accuracy check

This method generally yields 95–100% recovery on organic nitrogen standards. For proof of accuracy use Primary Standards for Kjeldahl Nitrogen.

1. Prepare one or more of the following three solutions. Each preparation is for an equivalent 25-mg/L N standard. Use the deionized water included in the kit or water that is free of all organic and nitrogen-containing species.
 - a. Weigh 0.3379 g of Ammonium p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
 - b. Weigh 0.4416 g of Glycine p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
 - c. Weigh 0.5274 g of Nicotinic p-Toluenesulfonate (PTSA). Dissolve in a 1000-mL volumetric flask with deionized water. Add deionized water to the 1000-mL mark.
2. Analyze each of these solutions using the test procedure above. Calculate the percent recovery for each using this formula. Refer to the *Percent recovery* table for more information.

$$\% \text{ recovery} = \frac{\text{measured concentration}}{25} \times 100$$

Nitrogen, Total

Refer to the *Percent recovery* table.

Table 4 Percent recovery

Compound	Lowest Expected % Recovery
Ammonia-PTSA	95%
Glycine-PTSA	95%
Nicotinic-PTSA	95%

Analysts have found Ammonia-PTSA to be the most difficult to digest. Other compounds may yield different percent recoveries.

Standard additions method (sample spike)

Required for accuracy check:

- Ammonia Nitrogen Standard Solution, 1000-mg/L as $\text{NH}_3\text{-N}$
- Ampule breaker
- TenSette Pipet
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADDITIONS
DR 2800	OPTIONS>MORE>STANDARD ADDITIONS
DR 2700	OPTIONS>MORE>STANDARD ADDITIONS
DR/2500	OPTIONS>STANDARD ADDITIONS
DR/2400	OPTIONS>STANDARD ADDITIONS

3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Open the standard solution ampule.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
6. Follow the *Persulfate digestion method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method

Note: Refer to the instrument user manual for specific software navigation instructions.

Required for accuracy check:

- 10-mg/L ammonia nitrogen standard solution
1. Substitute 2 mL of a 10-mg/L ammonia nitrogen standard solution in place of the sample. Follow the *Persulfate digestion method* test procedure.

Nitrogen, Total

- To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADJUST
DR 2800	OPTIONS>MORE>STANDARD ADJUST
DR 2700	OPTIONS>MORE>STANDARD ADJUST
DR/2500	OPTIONS>STANDARD ADJUST
DR/2400	OPTIONS>STANDARD ADJUST

- Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity— Δ Concentration per 0.010 Δ Abs
350	DR 5000	10 mg/L NH ₃ -N	9.6–10.4 mg/L N	0.5 mg/L N
350	DR 2800	10 mg/L NH ₃ -N	9.6–10.4 mg/L N	0.5 mg/L N
350	DR 2700	10 mg/L NH ₃ -N	9.6–10.4 mg/L N	0.5 mg/L N
350	DR/2500	10 mg/L NH ₃ -N	9.0–11.0 mg/L N	0.5 mg/L N
350	DR/2400	10 mg/L NH ₃ -N	9.0–11.0 mg/L N	0.5 mg/L N

Summary of method

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.

Consumables and replacement items

Required reagents

Description	Unit	Catalog number
Test 'N Tube™ Total Nitrogen Reagent Set, LR	50 vials	2672245

Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
DRB200 Reactor, 110 V, 15x16 mm	1	each	LTV082.53.40001
DRB200 Reactor, 220 V, 15x16 mm	1	each	LTV082.52.40001
Funnel, micro	1	each	2584335
Pipet, TenSette®, 1.0 to 10.0 mL	1	each	1970010
Pipet Tips, for TenSette Pipet 19700-10	2	50/pkg	2199796
Test Tube Cooling Rack	1–3	each	1864100
Finger Cots	2	2/pkg	1464702

Nitrogen, Total
Page 7 of 8

Nitrogen, Total

Recommended standards

Description	Unit	Catalog number
Ammonia Nitrogen Standard Solution, 1000-mg/L NH ₃ -N	1 L	2354153
Ammonia Nitrogen Standard Solution, 10-mg/L NH ₃ -N	500 mL	15349
Primary Standard Set, for Kjeldahl Nitrogen	set of 3	2277800
Wastewater Mixed Inorganic Standard for NH ₃ -H, NO ₃ -N, PO ₄ , COD, SO ₄ , TOC	500 mL	2833149
Water, deionized	500 mL	27249
Water, organic-free	500 mL	2641549

Optional reagents and apparatus

Description	Unit	Catalog number
Balance, analytical, 80 g capacity, 115 VAC	each	2936701
Cylinder, mixing with stopper, 50 mL	each	2088641
Flask, volumetric, Class A, 1000 mL	each	1457453
Pipet, TenSette, 0.1 to 1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet tips for TenSette Pipet 1970001	1000/pkg	2185628
Pipet tips for TenSette Pipet 1970010	250-pkg	2199725
Sodium Hydroxide, 5 N	50 mL	245026
Sulfuric Acid, concentrated	500 mL	97949
PourRite® Ampule breaker, 2-mL	each	2484600
Voluette® Ampule breaker 10 mL	each	2196800
Ammonia Nitrogen Standard Solution, 1-mg/L NH ₃ -N	500 mL	189149
Ammonia Nitrogen Standard Solution, 100-mg/L NH ₃ -N	500 mL	2406549
Ammonia Nitrogen Standard Solution, 2-mL PourRite Ampule, 50 mg/L	20/pkg	1479120
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 10 mg/L	16/pkg	1479110
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 150 mg/L	16/pkg	2128410
Ammonia Nitrogen Standard Solution, 10-mL Voluette Ampules, 160 mg/L	16/pkg	2109110



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

© Hach Company, 2007. All rights reserved. Printed in the U.S.A.

Updated February 2008, Edition 5

Nitrate

DOC316.53.01069

Cadmium Reduction Method **Method 8171**
MR (0.1 to 10.0 mg/L NO₃⁻-N) **Powder Pillows or AccuVac® Ampuls**

Scope and Application: For water, wastewater and seawater

! Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.

A deposit of unoxidized metal will remain at the bottom of the cell after the NitraVer® 5 dissolves. The deposit will not affect results.

This method is technique-sensitive. Shaking time and technique influence color development. For most accurate results, make successive tests on a 10.0-mg/L Nitrate Nitrogen Standard solution. Adjust shaking times to obtain the correct result.

Rinse the sample cell immediately after use to remove all cadmium particles. Retain the used sample for proper hazardous waste disposal for cadmium.

Prepared samples will contain cadmium and must be disposed of according to Federal, State and local hazardous waste regulations. Refer to the current MSDS for safe handling and disposal instructions.

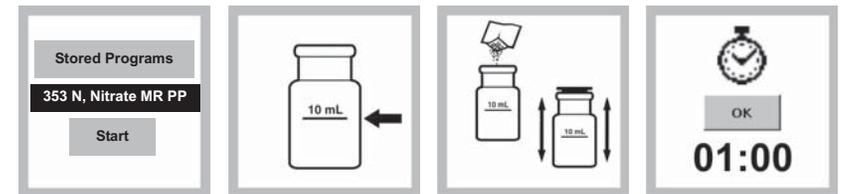
Nitrate

Collect the following items:

Description	Quantity
Powder Pillow Test:	
NitraVer® 5 Nitrate Reagent Powder Pillow	1
Sample Cells (see <i>Instrument-specific information</i>)	2
Stopper, Neoprene #2, solid	2
AccuVac Test:	
Collect at least 40 mL of sample in a 50-mL beaker	40 mL
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1
Beaker, 50-mL (AccuVac test)	1
Sample Cell for blank (see <i>Instrument-specific information</i>)	1

See *Consumables and replacement items* for reorder information.

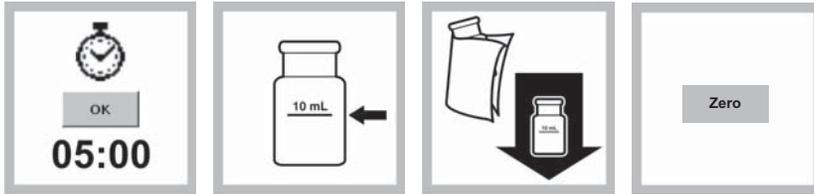
Cadmium reduction method for powder pillows



- 1. Select the test.**
Insert an adapter if required (see *Instrument-specific information*).
Refer to the user manual for orientation.
- 2. Fill a sample cell with 10 mL of sample.**
- 3. Prepared Sample:**
Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow. Insert a stopper into the cell.
- 4. Start the instrument timer.**
A one-minute reaction period will begin.
Shake the cell vigorously until the timer expires.
Note: Some solid material will not dissolve.

Nitrate

Cadmium reduction method for powder pillows (continued)



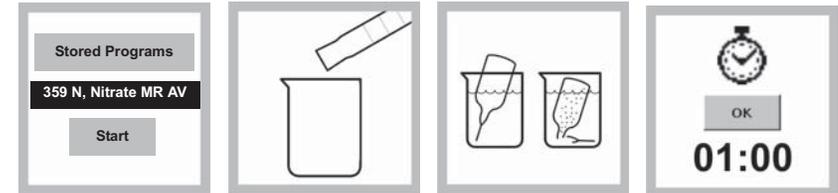
5. When the timer expires, start the timer again.
A five-minute reaction period will begin.
An amber color will develop if nitrate is present.
6. **Blank Preparation:** When the timer expires, fill a second sample cell with 10 mL of sample.
7. Wipe and insert the blank into the cell holder.
8. **ZERO** the instrument.
The display will show:
0.0 mg/L NO₃⁻-N



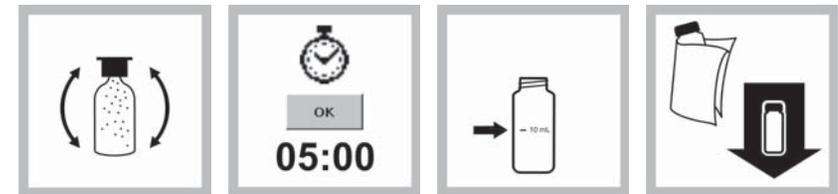
9. Within two minutes after the timer expires, wipe and insert the prepared sample into the cell holder.
10. **READ** the results in mg/L 0.0 mg/L NO₃⁻-N.
Refer to the user manual to display other chemical forms.

Nitrate

Cadmium reduction method for AccuVac® Ampuls



1. Select the test.
Insert an adapter if required (see *Instrument-specific information*).
Refer to the user manual for orientation.
2. **Prepared Sample:** Collect at least 40 mL of sample in a 50-mL beaker.
3. Fill a NitraVer 5 Nitrate AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills completely. Place a stopper over the Ampul tip.
4. Start the instrument timer.
A one-minute reaction period will begin.



5. Invert the Ampul 48–52 times as the timer counts down.
6. When the timer expires, start the timer again.
A five-minute reaction period will begin. An amber color will develop if nitrate is present.
7. **Blank Preparation:** When the timer expires, fill a round sample cell with 10 mL of sample.
8. Wipe the blank and insert it into the cell holder.



9. **ZERO** the instrument.
The display will show:
0.0 mg/L NO₃⁻-N
10. Within two minutes after the timer expires, wipe the Ampul and insert it into the cell holder.
11. **READ** the results in mg/L NO₃⁻-N.

Nitrate

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Chloride	Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride concentrations (seawater) but a calibration must be done using standards spiked to the same chloride concentration (see <i>Seawater calibration</i>).
Ferric iron	Interferes at all levels
Nitrite	Interferes at all levels Compensate for nitrite interference as follows: 1. Add 30-g/L Bromine Water ¹ drop-wise to the sample until a yellow color remains. 2. Add one drop of 30-g/L Phenol Solution ¹ to destroy the color. 3. Proceed with Step 2 of the test. Report the results as total nitrate and nitrite.
pH	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment.
Strong oxidizing and reducing substances	Interfere at all levels

¹ See *Optional reagents and apparatus*.

Seawater calibration

Chloride concentrations above 100 mg/L will cause low results. To perform this test in water with high interference level, calibrate the water using standards spiked to the same chloride concentrations as the required samples. To prepare calibration standards containing 0.06, 0.1, 0.3 and 0.4 mg/L nitrate as NO₃-N:

- Prepare a 1 L volume of chloride water that matches the concentration of the samples, using the following equation:
 - Add necessary Chloride concentration (g/L) x (1.6485) = g of ACS grade NaCl to 1 L of deionized water. (

Note: 18.8 g/L is a typical seawater chloride concentration.
 - Mix this solution thoroughly to make sure that it is a homogeneous solution. Use this water as the dilution water instead of the deionized water when preparing the nitrate standards.
- Use Class A glassware or a Tensette Pipet to pipet 0.6, 1, 3 and 4 mL of the 10 mg/L Nitrogen-Nitrate as NO₃-N (NIST) Standard Solution (Catalog Number 30749) into four different 100 mL Class A volumetric flasks.
- Dilute to the mark with the prepared chloride water. Mix thoroughly.
- Use the prepared chloride water for the 0-mg/L nitrate as NO₃-N standard.

Sample collection, preservation and storage

- Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, store samples in clean plastic or glass bottles for up to 24 hours at 4 °C. To preserve samples for longer periods, add 2 mL of Concentrated Sulfuric Acid (H₂SO₄)^{*} per liter and store at 4 °C. The results are reported as total nitrate and nitrite.
- Before analysis, warm the sample to room temperature and adjust the pH to 7 with 5.0 N Sodium Hydroxide Standard Solution*. Do not use mercury compounds as preservatives.

* See *Optional reagents and apparatus*.

Nitrate

- Correct the test result for volume additions by dividing the total volume (acid + base + sample) by the original sample volume and multiplying the test result by this factor.

Accuracy check

Standard additions method (sample spike)

Required for accuracy check:

- Nitrate Nitrogen Standard, 100-mg/L NO₃⁻-N
- TenSette Pipet and Pipet Tips

- After reading test results, leave the sample cell (unspiked sample) in the instrument.
- Select standard additions from the instrument menu:

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADDITIONS
DR 2800	OPTIONS>MORE>STANDARD ADDITIONS
DR 2700	OPTIONS>MORE>STANDARD ADDITIONS
DR/2500	OPTIONS>STANDARD ADDITIONS
DR/2400	OPTIONS>STANDARD ADDITIONS

- Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
- Open the standard solution bottle.
- Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of standard to three 10-mL portions of fresh sample.
- Follow the *Cadmium reduction method for powder pillows* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
- Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard additions method for AccuVac Ampuls (sample spike)

Required for accuracy check:

- 500 mg/L Nitrate Nitrogen Ampule Standard Solution
- Ampule breaker
- TenSette Pipet and Pipet Tips
- Mixing cylinder, 50-mL (3)

- Fill three mixing cylinders each with 50-mL of sample and spike with 0.1 mL, 0.2 mL and 0.3 mL of 500 mg/L Nitrate Nitrogen Ampule Standard Solution.
- Transfer 40 mL from each of the three mixing cylinders to three 50-mL beakers.
- Analyze each standard addition sample as described in the *Cadmium reduction method for AccuVac® Ampuls*.
- Accept each standard additions reading. Each addition should reflect approximately 100% recovery. Standard solution method

Note: Refer to the instrument user manual for specific software navigation instructions.

Nitrate

Required for accuracy check:

- 5.0-mg/L Nitrate Nitrogen Standard Solution (prepared)
- 100-mg/L Nitrate Nitrogen Standard
- Deionized water
- 100-mL volumetric flask
- 5-mL Volumetric pipet
- TenSette Pipet and Pipet Tips

1. Prepare a 5.0-mg/L nitrate nitrogen standard solution as follows:
 - a. Pipet 5.0 mL of 100-mg/L Nitrate Nitrogen Standard, into a 100-mL volumetric flask.
 - b. Dilute to the mark with deionized water. Mix well.
2. Use this 5.0 mg/L nitrate nitrogen standard solution in place of the sample. Follow the *Cadmium reduction method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADJUST
DR 2800	OPTIONS>MORE>STANDARD ADJUST
DR 2700	OPTIONS>MORE>STANDARD ADJUST
DR/2500	OPTIONS>STANDARD ADJUST
DR/2400	OPTIONS>STANDARD ADJUST

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
353	DR 5000	5.0 mg/L NO ₃ ⁻ -N	4.8–5.2 mg/L NO ₃ ⁻ -N	0.04 mg/L NO ₃ ⁻ -N
	DR 2800			
	DR 2700			
	DR/2500			
	DR/2400			
359	DR 5000	5.0 mg/L NO ₃ ⁻ -N	4.6–5.4 mg/L NO ₃ ⁻ -N	0.05 mg/L NO ₃ ⁻ -N
	DR 2800			
	DR 2700			
	DR/2500			
	DR/2400			

Nitrate
Page 7 of 8

Nitrate

Summary of method

Cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. Test results are measured at 400 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitraVer® 5 Nitrate Reagent Powder Pillows (for 10 mL sample) OR	1	100/pkg	2106169
NitraVer® 5 Nitrate Reagent AccuVac® Ampul	1	25/pkg	2511025

Required apparatus (powder pillows)

Description	Quantity/Test	Unit	Catalog number
Stopper, Neoprene, solid, size no. 2	2	12/pkg	1480802

Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H

Recommended standards

Description	Unit	Catalog number
Mixed Parameter Drinking Water Standard, for F, NO ₃ ⁻ -N, PO ₄ , SO ₄	500 mL	2833049
Nitrate Nitrogen Standard Solution, 100-mg/L NO ₃ ⁻ -N	500 mL	194749
Nitrate Nitrogen Standard Solution, 500 mg/L NO ₃ ⁻ -N, 10-mL ampules	16/pkg	1426010
Water, deionized	4 L	27256

Optional reagents and apparatus

Description	Unit	Catalog number
Bromine Water, 30-mg/L	29 mL	221120
Cylinder, mixing, 50 mL	each	2088641
Flask, volumetric, 100-mL	each	1457442
Pipet, TenSette, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001	1000/pkg	2185628
Pipet, volumetric, 5.00 mL	each	1451537
Pipet Filler, safety bulb	each	1465100
Phenol Solution, 30-g/L	29 mL	211220
5.0 N Sodium Hydroxide Standard Solution	1 L	245053
Sulfuric Acid, concentrated	500 mL	97949



FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – Call toll-free 800-227-4224
Outside the U.S.A. – Contact the HACH office or distributor serving you.
On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

© Hach Company, 2007. All rights reserved. Printed in the U.S.A.

Updated February 2008, Edition 5

Nitrite

DOC316.53.01074

USEPA¹ Diazotization

Method 8507

LR (0.002 to 0.300 mg/L NO₂⁻-N)

Powder Pillows or AccuVac[®] Ampuls

Scope and Application: For water, wastewater and seawater

¹ USEPA approved for wastewater analysis, Federal Register, 44(85), 25505 (May 1, 1979)

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 1 Instrument-specific information

Instrument	Powder pillows			AccuVac Ampuls	
	Sample cell	Cell orientation	Adapter	Sample cell	Adapter
DR 5000	2495402	Fill line faces user	A23618	2427606	A23618
DR 2800	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR 2700	2495402	Fill line faces right	—	2122800	LZV584 (C)
DR/2500	2427606	—	—	2427606	—
DR/2400	2427606	—	—	2427606	—

Before starting the test:

For more accurate results, determine a reagent blank value for each new lot of reagent. Follow the procedure using deionized water instead of the sample.

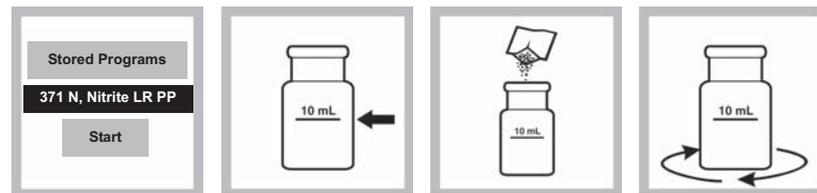
Collect the following items:

Description	Quantity
Powder Pillow Test:	
NitriVer [®] 3 Nitrite Reagent Powder Pillows	1
Sample Cells (see <i>Instrument-specific information</i>)	2
AccuVac Test:	
NitriVer [®] 3 Nitrite Reagent AccuVac [®] Ampul.	1
Beaker, 50-mL	1
Sample Cell (see <i>Instrument-specific information</i>)	1

See *Consumables and replacement items* for reorder information.

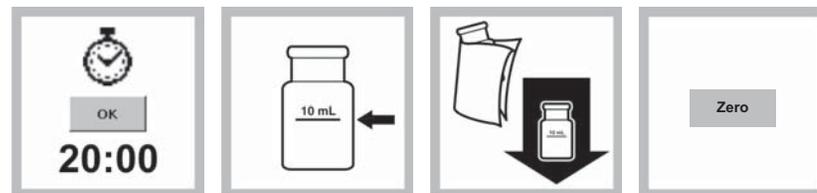
Nitrite

Diazotization method for powder pillows



1. Select the test. Insert an adapter if required (see *Instrument-specific information*).
2. Fill a sample cell with 10 mL of sample.
3. **Prepared Sample:** Add the contents of one NitriVer 3 Nitrite Reagent Powder Pillow.
4. Swirl to dissolve. A pink color will develop if nitrite is present.

Refer to the user manual for orientation.



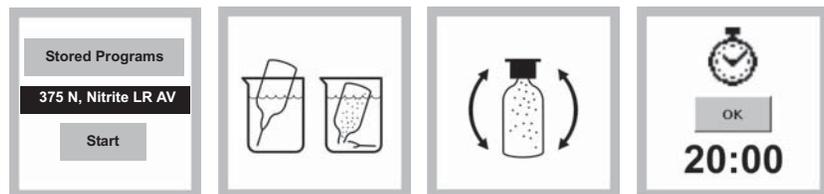
5. Start the instrument timer. A 20-minute reaction period will begin.
6. **Blank Preparation:** When the timer expires, fill a second sample cell with 10 mL of sample.
7. Wipe the blank and insert it into the cell holder.
8. **ZERO** the instrument. The display will show: 0.000 mg/L NO₂⁻-N



9. Wipe the prepared sample and insert it into the cell holder.
10. **READ** the results in mg/L NO₂⁻-N.

Nitrite

Diazotization method for AccuVac® Ampuls



1. Select the test. Insert an adapter if required (see *Instrument-specific information*). Refer to the user manual for orientation.
2. **Prepared Sample:** Collect at least 40 mL of sample into a 50-mL beaker. Fill a NitrVer 3 Nitrite AccuVac® Ampul with sample. Keep the tip immersed while the Ampul fills.
3. Invert the Ampul several times to mix. A pink color will develop if nitrite is present.
4. Start the instrument timer. A 20-minute reaction period will begin.



5. **Blank Preparation:** When the timer expires, fill a sample cell with 10 mL of sample.
6. Wipe the blank and insert it into the cell holder. **ZERO** the instrument. The display will show: 0.000 mg/L NO₂⁻-N.
7. Wipe the Ampul and insert it into the cell holder. **READ** the results in mg/L NO₂⁻-N.

Nitrite

Interferences

Table 2 Interfering substances

Interfering substance	Interference level
Antimonous ions	Interfere by causing precipitation
Auric ions	Interfere by causing precipitation
Bismuth ions	Interfere by causing precipitation
Chloroplatinate ions	Interfere by causing precipitation
Cupric ions	Cause low results
Ferric ions	Interfere by causing precipitation
Ferrous ions	Cause low results
Lead ions	Interfere by causing precipitation
Mercurous ions	Interfere by causing precipitation
Metavanadate ions	Interfere by causing precipitation
Nitrate	Very high levels of nitrate (>100 mg/L nitrate as N) appear to undergo a slight amount of reduction to nitrite, either spontaneously or during the course of the test. A small amount of nitrite will be found at these levels.
Silver ions	Interfere by causing precipitation
Strong oxidizing and reducing substances	Interferes at all levels

Seawater calibration

Chloride concentrations above 100 mg/L will cause low results. To perform this test in water with high interference level, calibrate the water using standards spiked to the same chloride concentrations as the required samples. To prepare calibration standards containing 0.06, 0.1, 0.3 and 0.4 mg/L nitrate as NO₃⁻-N:

1. Prepare a 1 L volume of chloride water that matches the concentration of the samples, using the following equation:
 - a. Add necessary Chloride concentration (g/L) x (1.6485) = g of ACS grade NaCl to 1 L of deionized water. (

Note: 18.8 g/L is a typical seawater chloride concentration.
 - b. Mix this solution thoroughly to make sure that it is a homogeneous solution. Use this water as the dilution water instead of the deionized water when preparing the nitrate standards.
2. Use Class A glassware or a Tensette Pipet to pipet 0.6, 1, 3 and 4 mL of the 10 mg/L Nitrogen-Nitrate as NO₃-N (NIST) Standard Solution (Catalog Number 30749) into four different 100 mL Class A volumetric flasks.
3. Dilute to the mark with the prepared chloride water. Mix thoroughly.
4. Use the prepared chloride water for the 0-mg/L nitrate as NO₃-N standard.

Sample collection, preservation and storage

- Collect samples in clean plastic or glass bottles.
- Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours.
- Warm to room temperature before running the test.
- Do not use acid preservatives.

Nitrite

Accuracy check

Standard solution method

Note: Refer to the instrument user manual for specific software navigation instructions.

1. Preparing nitrite standards is difficult. Use the standard preparation instructions in *Standard Methods for the Examination of Water and Wastewater*, Method 4500—NO₂-B. Prepare a 0.150-mg/L standard.
2. Use the 0.150 mg/L solution in place of the sample. Follow the *Diazotization method for powder pillows* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software.

Instrument	Navigate to:
DR 5000	OPTIONS>MORE>STANDARD ADJUST
DR 2800	OPTIONS>MORE>STANDARD ADJUST
DR 2700	OPTIONS>MORE>STANDARD ADJUST
DR/2500	OPTIONS>STANDARD ADJUST
DR/2400	OPTIONS>STANDARD ADJUST

4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Instrument	Standard	Precision 95% Confidence Limits of Distribution	Sensitivity Concentration change per 0.010 Abs change
371	DR 5000	0.150 mg/L NO ₂ ⁻ -N	0.147–0.153 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR 2800	0.150 mg/L NO ₂ ⁻ -N	0.147–0.153 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR 2700	0.150 mg/L NO ₂ ⁻ -N	0.147–0.153 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR/2500	0.150 mg/L NO ₂ ⁻ -N	0.146–0.154 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR/2400	0.150 mg/L NO ₂ ⁻ -N	0.146–0.154 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
375	DR 5000	0.150 mg/L NO ₂ ⁻ -N	0.147–0.153 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR 2800	0.150 mg/L NO ₂ ⁻ -N	0.147–0.153 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR 2700	0.150 mg/L NO ₂ ⁻ -N	0.147–0.153 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR/2500	0.150 mg/L NO ₂ ⁻ -N	0.140–0.160 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N
	DR/2400	0.150 mg/L NO ₂ ⁻ -N	0.140–0.160 mg/L NO ₂ ⁻ -N	0.002 mg/L NO ₂ ⁻ -N

Summary of method

Nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present. Test results are measured at 507 nm.

Nitrite

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
NitriVer® 3 Nitrite Reagent Powder Pillows	1	100/pkg	2107169
OR			
NitriVer® 3 Nitrite Reagent AccuVac® Ampul	1	25/pkg	2512025

Required apparatus (AccuVac)

Description	Quantity/Test	Unit	Catalog number
Beaker, 50-mL	1	each	50041H

Recommended standards, reagents and apparatus

Description	Unit	Catalog number
Balance, Analytical	each	2936701
AccuVac ampules, for blanks	25/pkg	2677925
AccuVac Snapper	each	2405200
AccuVac Drainer	each	4103600
Handbook, Standard Methods for the Examination of Water and Wastewater	each	—
Sodium Nitrite, ACS	454 g	245201
Water, deionized	4 L	27256



Section 6

Instrument and Probe Maintenance





Instrument and Probe Maintenance

Wastewater Treatment Lab

TDEC - Fleming Training Center 1



Instruments

- Whenever you have any maintenance done on an instrument, you should keep records on it
 - After maintenance, you should check calibration
- Keep records on calibrations

TDEC - Fleming Training Center 2



Instruments

- Good idea to have separate book for each instrument
- Keep instruments clean so they work best
- Always have replacement parts on hand
 - Bulbs for turbidimeters and spectrophotometers

TDEC - Fleming Training Center 3



pH Meters

- Good recommendation: water industry should not use gel filled probes
- Calibrate DAILY with fresh buffers
- Three buffers are better than two
 - If you use two buffers, you should use buffers that bracket your normal readings
 - 10 buffer degrades faster than 4 and 7

TDEC - Fleming Training Center 4



pH Probes

- Store probes in slightly acidic solution
 - Can be bought premixed
 - pH 4 buffer works also
- Don't store in distilled water or finished water
 - Becomes slow to respond
- pH electrode (glass ball) is porous for H⁺ to migrate through
- Can clean with dilute hydrochloric acid (HCl) is slow response for 30-60 seconds then rinse with distilled water for 15-20 minutes
 - Follow manufacturer's recommendation

TDEC - Fleming Training Center 5

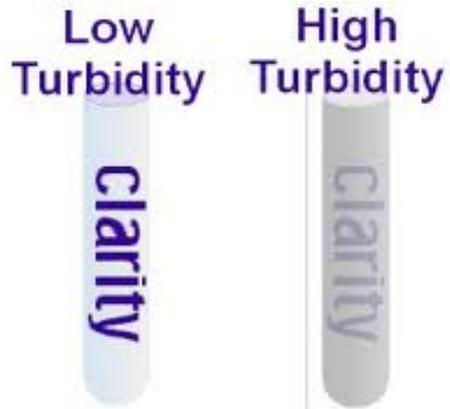


pH Probes

- Replace annually
 - Good practice to put date on probe
 - Have back up probe at all times
- For fillable probes, if crystals form in reference electrode area
 - Shake out solution
 - Fill with distilled water until crystals dissolve
 - Dump out then refill with correct solution

TDEC - Fleming Training Center 6

Section 7
Turbidity



Turbidity

Laboratory Workshop



TDEC - Fleming Training Center

1

Turbidity

- ❑ A measure of the clarity of water
- ❑ It is an expression of the optical property that causes light to be scatter and absorbed in water
- ❑ It is caused by particulate, such as silt, clay, organic matter, algae and other microorganisms
- ❑ Amount of light absorbed is proportional to the concentration of particulate in the sample

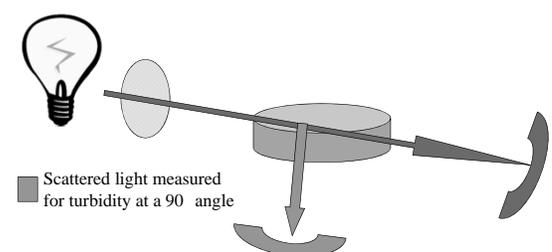
TDEC - Fleming Training Center 2

Turbidity

- ❑ Caused by suspended and colloidal matter in water
- ❑ It is expression of light that is scattered or absorbed through a sample
- ❑ Does not indicate the number or size of particles in a sample
- ❑ General indicator of overall effluent water quality and a good process control test for operator

TDEC - Fleming Training Center 3

Turbidimeters



- Scattered light measured for turbidity at a 90° angle
- Light source from tungsten lamp passing through three precisely aligned lenses, the light is focused in a narrow, collimated beam

Importance

- ❑ Supports growth of microorganisms
- ❑ Reduces effectiveness of chlorination
- ❑ Interferes with chemical and microbiological analysis
- ❑ Is unacceptable for aesthetic reasons
- ❑ Is related to coagulation and filtration
- ❑ Is unacceptable for most industrial water

TDEC - Fleming Training Center 5

Measuring

- ❑ Use an instrument for measuring and comparing turbidity of liquids
- ❑ Nephelometers are instruments which measure turbidity by comparing the amount of light in a sample to the amount of light scattered by a standard
- ❑ The amount of scattered light is measured and converted to units of turbidity or NTU's (Nephelometric Turbidity Units)

TDEC - Fleming Training Center 6

Instruments



Measuring Notes

- ❑ Always cap the sample cell to prevent spillage into instrument
- ❑ Close the sample compartment lid during measurement
- ❑ Do not leave sample cell in the cell compartment for extended periods of time
- ❑ Leave the instrument on 24 hours a day if instrument is used regularly

TDEC - Fleming Training Center

8

Measuring Notes

- ❑ Always use clean, scratch free sample cells and caps
- ❑ Always use silicone oil
- ❑ Measuring samples immediately to prevent changes in sample characteristics
- ❑ Remove air bubbles in sample cells
- ❑ Discard sample cells with scratches

TDEC - Fleming Training Center

9

Calibrations

- ❑ Use Gelex Secondary Turbidity Standards for periodic checks
- ❑ Primary Stable Cal Standards
 - Formazin Solution Primary Standards and Procedure for making solutions
- ❑ Record keeping requirements and recommendations for operators
- ❑ Calibrate at least quarterly

TDEC - Fleming Training Center

10



2100N LABORATORY TURBIDIMETER QUICK REFERENCE GUIDE

NEPHELOMETRIC MEASUREMENT PROCEDURE

1. Collect a representative sample in a clean container. Fill the sample cell to the line (approximately 30 mL). Take care to handle the sample cell by the top. Cap the sample cell. (Note: Instrument warm-up stabilization time with Ratio on is 30 minutes and with Ratio off is 60 minutes. Typical application is to leave the instrument on 24 hours a day.)
2. Hold the sample cell by the cap, and wipe to remove water spots and finger prints.
3. Apply a thin bead of silicone oil from the top to the bottom of the cell—just enough to coat the cell with a thin layer of oil. Using the oiling cloth provided, spread the oil uniformly. Then, wipe off the excess. The cell should appear nearly dry with little or no visible oil. (Note: See Section 2.3.2 Applying Silicone Oil in the instrument manual.)
4. Place the sample cell in the instrument cell compartment, and close the cell cover. (Note: For immediate update of the display, press **ENTER**.)
5. If necessary, insert the EPA filter. Select manual or automatic ranging by pressing the **RANGE** key.
6. Select the appropriate **SIGNAL AVERAGING** setting (on or off) by pressing the **SIGNAL AVG** key.
7. Select the appropriate **RATIO** setting (on or off) by pressing the **RATIO** key. (Note: Values >40 NTU require Ratio on.)
8. Select the appropriate measurement unit (NTU, EBC or NEPH) by pressing the **UNITS/EXIT** key.
9. Read and record the results.

CALIBRATION

Preparing Recommended Formazin Dilutions

Hach Company recommends use of 20-, 200-, 1000- and 4000-NTU Formazin standards for calibration of the Model 2100N Turbidimeter. Prepare all Formazin dilutions immediately before calibration, and discard the dilutions after use. While 4000-NTU stock solutions are stable for up to one year, diluted solutions deteriorate more rapidly. Prepare dilutions of 20, 200 and 1000 NTUs according to the directions in Table 2 (Formazin Standard Preparation) in Section 3 of the Instrument Manual. The dilution water also is used to make an initial blank measurement (refer to Section 3.2 Calibration in the Instrument Manual).

NOTE

The calibration is based on a first order linear equation consisting of up to three independent variables. Unpredictable results may occur if standards other than the recommended calibration points are used. The factory-suggested calibration points are those determined by Hach Company chemists and engineers to provide the best calibration accuracy. Use of standards other than those specified may result in less accurate calibrations.

Calibrating with Formazin Standards

The electronic and optical design of the 2100N Turbidimeter provides long-term stability and minimizes the need for frequent calibration. The three-detector ratiometric optical system compensates for electronic and optical system variations between calibrations. When data is used for USEPA reporting, recalibrate at least every 90 days, or as stipulated by the regulating authority. Refer to Section 3.2 Calibration in the Instrument Manual.

1. Fill a clean sample cell to the line (\approx 30 mL) with dilution water. Wipe the cell clean and apply a thin film of silicone oil.
2. Place the sample cell into the cell holder, and close the cell cover.
3. Press the **CAL** key. The S0 annunciator lights. The NTU value of the dilution water used in the previous calibration is displayed.
4. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. This result is stored and used to compensate for the turbidity of the dilution water.
5. The instrument automatically increments to the next standard, displays the expected NTU value (e.g., 20.00 NTU), and the S1 annunciator lights. Remove the sample cell from the cell holder.
6. Fill a clean sample cell to the line with well-mixed, 20-NTU Formazin standard. Wipe the sample cell clean, and apply a thin film of silicone oil on its surface. Place it into the cell holder, and close the cell cover.
7. Press the **ENTER** key. The display counts down from 60 to 0, and makes a measurement. The instrument automatically increments to the next standard, the display shows 200.0 NTU, and the S2 annunciator lights. Remove the sample cell from the instrument.
8. Fill a clean sample cell to the line with well-mixed, 200-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it into the cell holder, and close the cell cover. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The instrument automatically increments to the next standard, the display shows 1000 NTU, and the S3 annunciator lights. Remove the sample cell from the instrument.
9. Fill a clean sample cell to the line with well-mixed, 1000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and close the cell cover. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The display automatically increments to the next standard, the display shows 4000 NTU, and the S4 annunciator lights. Remove the sample cell from the instrument.
10. Fill a clean sample cell to the line with well-mixed, 4000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and close the cell cover. Press the **ENTER** key. The instrument counts down from 60 to 0, and then makes a measurement. The display automatically increments back to the dilution water standard. The S0 annunciator lights, and the previously measured value of the dilution water is displayed.
11. Press the **CAL** key. The instrument makes calculations based on the new calibration data, stores the new calibration and returns the instrument to the measurement mode.

Reviewing the Calibration Sequence

Press the **CAL** key and then use the **UP ARROW** key to scroll through the standards to review calibration data currently in effect. If the instrument is connected to a printer, pressing the **PRINT** key prints all of the calibration data in effect. Press the **UNITS/EXIT** key to return to the operating mode without altering the current calibration data.

Using Gelex® Secondary Turbidity Standards

Periodically, as experience or regulating authorities indicate, verify the instrument calibration using Gelex Secondary Standards. If the reading in the range of use is not within 5% of the standard's assigned value, recalibrate using Formazin primary standards (refer to Section 3.2.5 Using Gelex Secondary Turbidity Standards in the Instrument Manual).

1. Calibrate the instrument with Formazin (refer to Section 3.2 Calibration in the Instrument Manual).
2. Verify that the instrument is set for the NTU mode, Ratio on and Automatic Ranging.
3. Thoroughly clean the outside of the Gelex vials, and apply a thin coating of silicone oil.
4. Place the lowest NTU Gelex Standard in the sample compartment with the triangle on the vial aligned with the index mark on the instrument sample compartment. Close the sample cell cover.
5. Press the **ENTER** key. Record the value displayed. Remove the standard from the instrument, and mark this value on the vial with a water soluble marker.
6. Repeat steps 3 through 5 for the other Gelex standards.

NOTE

Reassign new values to the Gelex standards each time the instrument is calibrated with Formazin.

Turbidity

ERROR CODES

Error codes may result from instrument malfunction or operator error. **Errxx** error codes are cleared from the display by pressing the **ENTER** key. The meter continues operating in the error condition; a calibration in progress can be continued. Any calibration being calculated (at the time the message appears) is discarded; the old calibration is retained. *Table 1* lists the error codes displayed for specific conditions.

Table 1. Error Codes

Code	Probable Cause	Corrective Action
Err01	Dilution water calculated to be >0.5 NTU	Start calibration over with higher quality dilution water, or filter the water with a membrane filter before use.
Err02	Two calibration standards have the same value, or their difference is less than 60.0 NTU. Standard 1 is too low (<10 NTU)	Recheck preparation of standards and repeat calibration.
Err03	Low light error	Reinsert sample. Check that lamp is on. Dilution may be necessary.
Err04	Memory malfunction	Switch instrument off and back on with I/O. Call Hach Service.
Err05	A/D over-range	Contact Hach Service.
Err06	A/D under-range	Contact Hach Service.
Err07	Light leak	Contact Hach Service.
Err08	Bad lamp circuit	Contact Hach Service.
Err09	Printer timeout error	Check that external printer is properly connected. Check that external printer is selected (on-line).
Err10	System voltage out of range	Switch instrument off and back on with I/O. Call Hach Service.
Err11	System loop test error	Switch instrument off and back on with I/O. Call Hach Service.

Diagnostic Functions

The diagnostic mode accesses system function information that is useful primarily when the instrument function is in doubt. Hach service technicians use the information for precise troubleshooting, speeding repairs, and avoiding unnecessary service returns.

Access diagnostic information by pressing and holding the **RIGHT ARROW** key for 3 seconds. Use the **ARROW** keys to edit the display to read the diagnostic code number of interest. Press the **ENTER** key to display the diagnostic value. More information may be obtained by purchasing the instrument service manual, or contacting the service center nearest you.

Diagnostic Codes

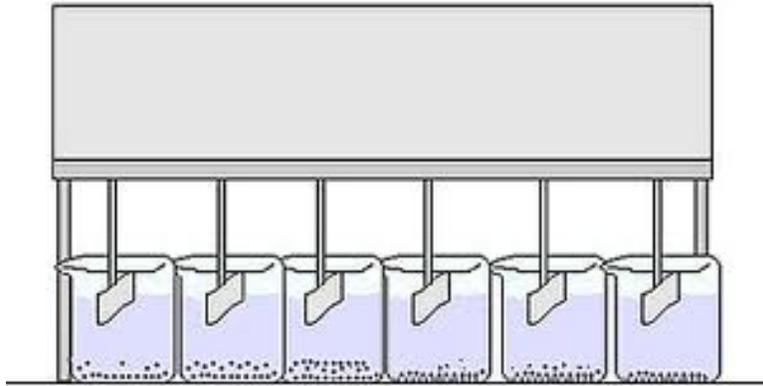
Code	Display	Description
00	bP on/bP of	Keyboard Beeper On/Off
01	FS Pr/SL Pr	Fast/Slow Print Device
21	Pr In	Printer Test
22	*	Display Test
23	*	Keyboard Test
24	*	Memory Test

Refer to *Table 6 Diagnostic Codes* in *Section 8 Troubleshooting* of the instrument manual for a list of diagnostic codes.



HACH COMPANY
 WORLD HEADQUARTERS
 P.O. BOX 389
 Loveland, Colorado 80539
 Telephone: (970) 669-3050
 FAX: (970) 669-2932

Section 8 Jar Testing



TDEC - Fleming Training Center 1

JAR TESTING

Wastewater Lab

Solids Removal Using Chemicals

2

- Physical-chemical treatment
- Three step process, must occur in proper sequence
 - Coagulation Phase: Chemicals are added to the wastewater and rapidly mixed forming "pinpoint floc"
 - Flocculation Phase: Gentle mixing to produce larger, denser floc particles that settle rapidly
 - Liquid/solids separation is almost always conventional sedimentation by gravity settling although air flotation is used occasionally

TDEC - Fleming Training Center

Most Important Guidelines

3

- Provide enough energy to completely mix chemicals
- Control intensity of mixing during flocculation
- Control chemical(s) dose

TDEC - Fleming Training Center

Principles of Coagulation

4

- Coagulation involves both chemical (destabilization) and physical (mixing) processes.
- Coagulation produces solid particles that form strong enough to withstand shearing

TDEC - Fleming Training Center

Principles of Coagulation

5

- Van Der Waals Force: attractive force existing between particles that allows coagulation to occur
- Zeta Potential: Measurement in millivolts of particle strength surrounding solids. The more negative the number, the stronger particle charge and repelling forces between particles

TDEC - Fleming Training Center

Principles of Coagulation

6

- To achieve coagulation the electrostatic charges of particles must be modified to reduce Zeta Potential
- Add chemicals that have charge opposite of the suspended solids
- Wastewater tends to have a negative ionic charge and pH of 6.5

TDEC - Fleming Training Center

Flocculation Process

7

- Most precise application of flocculation is in the preparation of secondary effluents for tertiary filtration use to "polish" the effluent.
- Simple coagulation not sufficient to enlarge suspended solids particles enough to meet pre-filtration conditioning requirements.
- If suspended solids less than 15 mg/L leaving the secondary treatment process polymers use

TDEC - Fleming Training Center

Flocculation Process

8

- Selection of polymers depends on pH, conductivity, type and concentration of suspended solids, particle size ranges, type and amount of coagulant(s) added, and next stage in treatment process
- Most important to have sufficient paddle speeds during flocculation to keep floc from settling and not shearing (adjustable speed drives)

TDEC - Fleming Training Center

Chemicals Used to Improve Settling

9

- Aluminum Sulfate (Dry or Liquid)
- Ferric Chloride
- Lime
- Polymers

TDEC - Fleming Training Center

Aluminum Sulfate

10

- Very corrosive when mixed with water
- Supports bacterial growth and/or cause sludge deposits in feed lines
- Reduces alkalinity in water being treated during the coagulation process
- Hydrated lime, soda ash, or caustic soda may be required to adjust alkalinity for coagulation process to occur
- A 1% solution will have a pH of 3.5
- Alkalinity adjustment: 1 mg/L of chemical for alkalinity adjustment when using Alum

TDEC - Fleming Training Center

Ferric Chloride

11

- Very Corrosive
- Will leave stains
- Temperature rises as chemical dissolves
- Crystallizes in temperatures below -1 °C
- Positive displacement pumps should be used for accuracy
- Will lower pH

TDEC - Fleming Training Center

Lime

12

- Use to coagulate solids or adjust pH to improve coagulation
- Very irritating to skin, eyes, and mucous membranes
- High heat is generated when water is added to chemical
- Quicklime less expensive but slaking required

TDEC - Fleming Training Center

Polymers

13

- High molecular weight organic compounds
- Natural or synthetic origin
- Wide range available
- May be applied alone or in combination with other chemicals
- Extensive laboratory tests
- Anionic, Nonionic, and Cationic Polymers

TDEC - Fleming Training Center

Selection of Chemicals and Dosages

14

- Three Step Process
 - Preliminary Screening
 - Dosage Testing
 - Full-Scale Trial
- Tested water treated must be freshly drawn samples from actual flow stream
- System performance optimization: regular testing of water upstream of chemical application

TDEC - Fleming Training Center

Jar Testing

15

- Basic guidelines:
 - Jar test volume 2 Liters
 - working chemical strengths should be 0.1% (1,000 mg/L)
 - Rapid mix at 140-160 RPM for 10-15 seconds for polymers: 3-5 minutes for aluminum and/or iron metal salts (Ferric Chloride)
 - Slow mix 15-20 RPM for 1-2 minutes for polymers; slow mix not used for aluminum and/or iron metal salts

TDEC - Fleming Training Center

Jar Testing

16

- Ranges for times represent normal operating conditions.
- Actual times based on composition of flow stream examined (detention and force gradients)

TDEC - Fleming Training Center

Water Bath

17

- Jars set in a rectangular tank with raw water circulating around them
- This is necessary for water that is not room temperature



TDEC - Fleming Training Center

Water Bath

18

- Determination for use of water bath:
 - Take a raw sample
 - Run jar test immediately
 - Take another sample
 - Let warm 5-10 degrees
 - Run same test and take note in difference



TDEC - Fleming Training Center

Solution Preparation

19

- Accuracy is critical - Small errors are compounded by large dilution
- Dilute solutions of 1 g/L or less of coagulants or polymers should be prepared daily

TDEC - Fleming Training Center

Solution Prep - Dry Products

20

- Dissolve 1 gram of a chemical that is 100% in 1000 ml of DI water
 - This is a 0.1% solution by weight or 1,000 mg/L
- In a 1 liter test beaker, 1 ml of the above equals 1 mg/L
- If the chemical is not 100%, then divide by the percent of the chemical available

TDEC - Fleming Training Center

Solution Prep - Dry Products

21

- Useful dilution for alum, iron salts, carbons and alkalis

TDEC - Fleming Training Center

Dry Alum

22

- Use a 1-L volumetric flask
- Dissolve 10 grams in 600 mL DI water
- Fill to the mark and mix
- Solution contains 10,000 mg/L
- Therefore, 1 mL of the stock solution added to a 2-L jar will equal a 5 mg/L alum dose



TDEC - Fleming Training Center

Solution Prep - Liquid Products

23

- To make a 10 g/L solution (1%) of a liquid product, divide 10 gm by specific gravity
 - This is the chemical to dissolve in 1000 ml DI water
 - In a 1 liter test beaker, 1 ml of the above equals 10 mg/L of liquid product
- For solutions like PACl

Remember, Jar Test beakers are usually 2L, therefore double dose

TDEC - Fleming Training Center

Solution Prep - Liquid Products

24

- For liquids sold on a dry basis, correct for concentration as follows
 - 10 gm/(sp.grav. x conc.)
 - In a 1 liter test beaker, 1 ml of the above equals 10 mg/L of liquid product
- For solutions like alum, ferric

Again, remember Jar Test beakers are usually 2L, therefore double dose

TDEC - Fleming Training Center

Solution Prep - Liquid Products

25

- Example:
 - Liquid aluminum sulfate is typically sold on a dry basis.
 - 48.5% $\text{Al}_2(\text{SO}_4)_3 \cdot 14 \text{H}_2\text{O}$ and specific gravity of 1.335
 - $10\text{g/L} / (1.335 \times 0.485) = 15.4 \text{ ml liquid alum for a 10 gram/liter dry basis solution.}$
 - 1 ml of this solution = 10 mg/L dry alum

TDEC - Fleming Training Center

Solution Prep - Liquid Products

26

- Micropipets dispense neat product
- Specific gravity must be accounted for (liquid chemicals weigh more than water, so 1 ml of product weighs more than 1 gram)
 - $\frac{\text{mg/L}}{\text{sp. grav.}} = \mu\text{L product / liter of test sol'n}$

TDEC - Fleming Training Center

Solution Prep - Liquid Products

27

- For dose on a dry active product basis, divide by concentration:
 - $\frac{\text{mg/L}}{\text{sp.grav.} \times \text{conc.}} = \mu\text{L product/L of test sol'n}$

TDEC - Fleming Training Center

Micro-pipeting

28

- Try to place dose on an object that can perch on the rim of the jar until you are ready to add all at the same time
 - Septas from TOC vials
 - Powder pillows



TDEC - Fleming Training Center

Jar Testing

29



- Must know volume of test jars and speed rates of stirrers prior to test
- Don't use laboratory grade chemicals
 - Use what you feed in plant

TDEC - Fleming Training Center

References

30

- Cal State Sacramento Advanced Waste Treatment Edition 3 Chapter 4. Solids Removal from Secondary Effluents
- Practical Manual of Wastewater Chemistry by Barbara A. Hauser, 1996, ISBN 1-57504-012-3, Ann Arbor Press, Inc.
- Water Treatment: Troubleshooting and Problem Solving, 1996, by Glenn M. Tillman ISBN 1-57504-001-8
- CRC Press LLC

TDEC - Fleming Training Center

Section 9
Oil and Grease



Oil & Grease

Liquid/Liquid
OR
Solid Phase

1

TDEC - Fleming Training Center

Currently Approved Methods

- EPA Method 1664A
- SM 5520B (20th edition only)
 - Solvent type limited to hexane only

2

TDEC - Fleming Training Center

Sample Collection/Preservation

- Glass wide mouth container (SM 5520A.3)
- Clean sample bottles with solvent
- Grab sample
- Use PTFE-lined caps or line cap with foil
- Collect (3)1 liter of samples (EPA 1664A)
- Acidify to pH < 2 with H₂SO₄ or HCL
- Cool to ≤6°C and analyze within 28 days

3

TDEC - Fleming Training Center

Summary of Method

- Two basic techniques used are:
 1. Liquid/Liquid extraction for partitioning followed by gravimetric determinations.
 2. Solid phase extraction followed by elution and then gravimetric determinations.

4

TDEC - Fleming Training Center

Significance of O & G

- If present in excessive amount, O&G may interfere with aerobic and anaerobic biological processes and lead to decreased wastewater treatment efficiency.
- Excess amounts of O&G if discharged may cause surface films and shoreline deposits leading to environmental degradation.

5

TDEC - Fleming Training Center

SOURCES OF O&G

- Animal fat
 - e.g. meat packaging plant & fish processing
- Vegetable Oils
 - e.g. food processing & restaurants
- Petroleum Products
 - e.g. refinery & asphalt

6

TDEC - Fleming Training Center

EPA METHOD 1664A

- Hexane extractable materials (HEM)
- Silica-Gel treatment (SGT-HEM) for petroleum products
- Measurements of materials that volatilize approximately below 85°C

7

TDEC - Fleming Training Center

Calibration & Standardization

- Calibrate the analytical balance at 2 mg and 100 mg using class "ASTM Class 1" weights
- Calibration shall be within $\pm 10\%$ at 2 mg and $\pm 0.5\%$ at 1000 mg. If values are not within these limits, the balance must be recalibrated

8

TDEC - Fleming Training Center

Liquid/Liquid Extraction Procedure

- Volume measurements
 - Mark sample bottle
 - Weigh sample and bottle
- Check pH of sample (<2) with stirring rod
- Transfer sample to a 2L separatory funnel
- Vigorous extraction for 2 minutes each with 30 mL hexane
- Extract sample three times; drain solvent with each extraction

9

TDEC - Fleming Training Center

Liquid/Liquid Extraction Procedure (continued)

- Filter each extraction through a funnel containing 10g of Na_2SO_4 , rinse with hexane and collect into a pre-weighed boiling flask
- Distill solvent at approximately 70°C at the distilling head
- Sweep out the flask for 15 seconds using a vacuum to remove any remaining solvent vapors

10

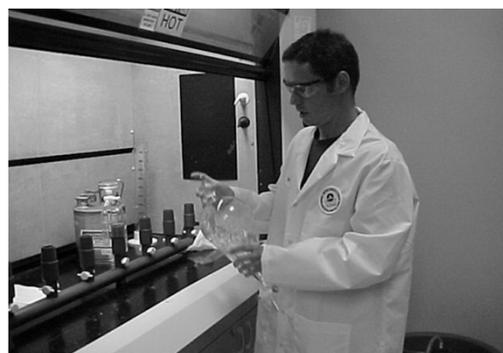
TDEC - Fleming Training Center

Liquid/Liquid Extraction Procedure (continued)

- Desiccate boiling flask containing residue for at least 30 minutes
- Weigh boiling flask and residue until measurement reaches a constant weight; within 4% or less than 0.5 mg whichever is less.

11

TDEC - Fleming Training Center



12

TDEC - Fleming Training Center

Solid Phase Extraction Procedure

- Volume measurements
 - Mark sample bottle
 - Weigh sample and bottle
- Check pH of sample (<2) with stirring rod
- Filter sample through solid phase disks
- Rinse sample bottle with 10 mL of hexane and pour over filter disks to elute sample
- Repeat this process 2 to 3 times with the 10 mL volumes of hexane

13

TDEC - Fleming Training Center

Solid Phase Extraction Procedure (continued)

- Collect solvent into a pre-weighed container
- Heat solvent at approximately 70°C and recover the condensate/distillate
- Sweep out the container for 15 seconds using a vacuum to remove any remaining solvent vapors
- Cool sample and desiccate prior to weighing
- Desiccate for 30 minutes and obtain constant weight

14

TDEC - Fleming Training Center



TDEC - Fleming Training Center

15

Calculations

$$Mg/L = \frac{final\ wt - initial\ weight(mg) \times 1000}{volume\ of\ sample(mL)}$$

16

TDEC - Fleming Training Center

Quality Control

- Initial demonstration of ability for accuracy and precision.
- Method Detection Limit (MDL) Study
- Blanks
- Method acceptance criteria found in Table I
 - Laboratory Control Sample (stearic acid & hexadecane)
 - Quality Control Sample (2nd Source)
 - Spikes
 - Matrix Spikes

17

TDEC - Fleming Training Center

Waste Management

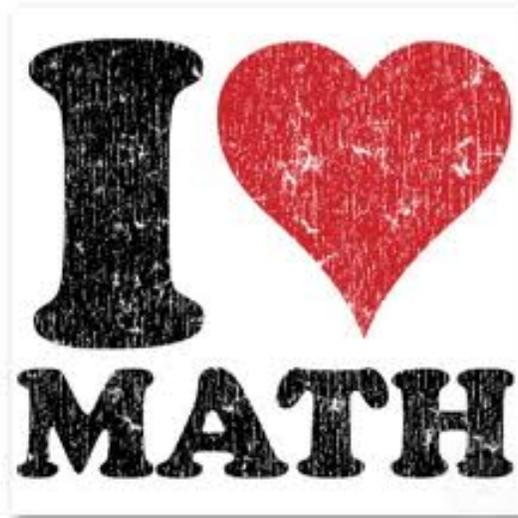
- It's the facilities responsibility to make sure the waste is recycled or disposed of properly



18

TDEC - Fleming Training Center

Section 10
Activated Sludge Math



Applied Math for Wastewater Treatment Activated Sludge

BOD or COD Loading, lbs/day

- This is the food part of the F/M ratio
- COD is sometimes used if there is a good correlation between it and BOD
- Loading guidelines for the 3 operational modes of Activated Sludge are:
 - High Rate
 - COD: greater than 1 lb COD/day/lb MLVSS under aeration
 - BOD: greater than 0.5 lb BOD/day/lb MLVSS under aeration
 - Conventional
 - COD: 0.5 to 1 lb COD/day/lb MLVSS under aeration
 - BOD: 0.25 to 0.5 lb BOD/day/lb MLVSS under aeration
 - Extended Aeration
 - COD: less than 0.2 lb COD/day/lb MLVSS under aeration
 - BOD: less than 0.1 lb BOD/day/lb MLVSS under aeration
- For untreated domestic wastewater, $BOD = (0.4 \text{ to } 0.8)(COD)$

Solids Inventory in the Aeration Tank, lbs. MLSS or lbs. MLVSS

- In an activated sludge system, the solids under aeration must be controlled
- The SS in aeration tank are the MLSS
- MLVSS is an estimate of the microorganism population in the aeration tank.
- The MLVSS is typically 70% of the MLSS, the remaining 30% are fixed (or inorganic) solids

Food to Microorganism Ratio

- In order for an Activated Sludge system to operate properly, there must be a balance between the food (BOD or COD) and bugs in the aeration tank (MLVSS).
- The F/M ratio is a process control calculation used in many activated sludge plants
- Best F/M depends on the type of activated sludge system and the wastewater characteristics
- The F/M ratio is calculated from the amount of BOD or COD applied each day and from the solids inventory in the aeration tank.
- Typical ranges for F/M (using BOD):
 - Conventional ranges are 0.2-0.4
 - Extended Aeration ranges are 0.05-0.15

Mean Cell Residence Time (MCRT), days

- Also called SRT, Solids Retention Time
- Approach used for solids control, adjust WAS to maintain MCRT
- Most desirable MCRT for a plant is determined experimentally
- Typical ranges are:
 - Conventional plants MCRT is 5-15 days
 - Extended aeration MCRT is 20-30 days
- MCRT based on suspended solids leaving the system and includes the aeration tank and final clarifier
- Also can determine the type of bugs that predominate and therefore the degree of nitrification that may occur
 - From AWT Table 2.6: MCRT needed to produce nitrified effluent as related to temp
 - 10°C – 30 days
 - 15°C – 20 days
 - 20°C – 15 days
 - 25°C – 10 days
 - 30°C – 7 days

Wasting Rates

- The amount of activated sludge wasted may vary from 1-20% of total incoming flow
- Expressed in lbs or gallons/day
- Wasting is the diverting of flow to primary clarifier, thickener, gravity belt thickener or aerobic or anaerobic digester

Applied Math for Wastewater Treatment Activated Sludge

BOD or COD Loading, lbs/day

1. The flow to an aeration tank is 850,000 gpd. If the BOD content of the wastewater entering the aeration tank is 225 mg/L, how many pounds of BOD are applied to the aeration tank daily?

2. The flow to an aeration tank is 1200 gpm. If the COD concentration of the wastewater is 155 mg/L, what is the COD loading rate in lbs/day?

Solids Inventory in the Aeration Tank, lbs. MLSS or lbs. MLVSS

3. An aeration basin is 120 ft long, 45 ft wide and holds wastewater to a depth of 12 ft. If the aeration basin has an MLSS concentration of 2150 mg/L, how many pounds of MLSS are under aeration?

- The aeration tank of a conventional activated sludge plant has an MLSS concentration of 2300 mg/L with a volatile solids content of 72%. If the volume of the aeration tank is 200,000 gallons, how many pounds of volatile solids are under aeration?

Food to Microorganism Ratio

- An activated sludge aeration tank receives a primary effluent flow of 1.6 MGD with a BOD concentration of 180 mg/L. The mixed liquor volatile suspended solids is 2200 mg/L and the aeration tank volume is 420,000 gallons. What is the current F/M ratio?
- The flow to a 195,000 gallon oxidation ditch is 365,000 gpd. The BOD concentration of the wastewater is 170 mg/L. If the MLSS concentration is 2550 mg/L with a volatile content of 70%, what is the F/M ratio?
- The desired F/M ratio of an extended aeration activated sludge plant is 0.5 lbs COD/lb. MLVSS. If the 3.0 MGD primary effluent flow has a COD of 172 mg/L, how many lbs of MLVSS should be maintained in the aeration tank?

Mean Cell Residence Time (MCRT), days

8. An activated sludge system has a total of 28,500 lbs of mixed liquor suspended solids. The suspended solids leaving the final clarifier in the effluent is 400 lbs/day. The pounds suspended solids wasted from the final clarifier is 2910 lbs/day. What is the solids retention time (MCRT), days?

9. Determine MCRT given the following information:

Aeration Tank = 1,400,000 gal
Final Clarifier = 105,000 gal
Flow = 3,000,000 gpd
WAS Pump Rate = 68,000 gpd

MLSS = 2650 mg/L
S.E. SS = 22 mg/L
CCSS = 1890 mg/L
WAS = 6050 mg/L

Wasting Rates

10. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.6 lbs BOD/lb MLVSS. It has been calculated that 3300 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 68%, how many lbs MLSS are desired in the aeration basin?

11. Using Constant MCRT: The desired MCRT for an activated sludge plant is 8.5 days. The secondary effluent flow is 3.16 MGD with a suspended solids content of 22 mg/L. There is a total of 32,100 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

Answers:

- | | |
|---------------------|---------------------------|
| 1. 1595 lbs BOD/day | 7. 8607 lbs MLVSS |
| 2. 2234 lbs COD/day | 8. 8.6 days |
| 3. 8691 lbs MLSS | 9. 8.2 days |
| 4. 2762 lbs MLVSS | 10. 8088 lbs MLSS desired |
| 5. 0.31 | 11. 3197 lbs to waste |
| 6. 0.18 | |

Applied Math for Wastewater Treatment
Activated Sludge
Extra Problems

BOD or COD Loading, lbs/day

1. The flow to an aeration basin is 880,000 gpd. If the BOD content of the wastewater entering the aeration basin is 240 mg/L, what is the lbs/day BOD loading?

2. The flow to the aeration basin is 2980 gpm. If the COD concentration of the wastewater is 160 mg/L, how many lbs of COD are applied to the aeration basin daily?

3. The BOD content of the wastewater entering an aeration basin is 165 mg/L. If the flow to the aeration basin is 3,240,000 gpd, what is the lbs/day BOD loading?

4. The daily flow to an aeration basin is 4,880,000 gpd. If the COD concentration of the influent wastewater is 150 mg/L, how many lbs of COD are applied to the aeration basin daily?

Solids Inventory in the Aeration Basin, lbs. MLSS or lbs. MLVSS

5. If the mixed liquor suspended solids concentration is 2110 mg/L and the aeration basin has a volume of 460,000 gallons, how many lbs of suspended solids are in the aeration basin?

6. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2420 mg/L. If the aeration basin is 90 ft long by 50 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

7. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2410 mg/L. If the aeration basin is 80 ft long by 40 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

8. An aeration basin is 110 ft long, 30 ft wide and has wastewater to a depth of 16 ft. If the aeration basin of this conventional activated sludge plant has a mixed liquor suspended solids (MLSS) concentration of 2740 mg/L, how many lbs of MLSS are under aeration?

9. An aeration basin is 110 ft long, 50 ft wide and has wastewater to a depth of 16 ft. If the mixed liquor suspended solids (MLSS) concentration in the aeration basin is 2470 mg/L with a volatile solids content of 73%, how many lbs of MLVSS are under aeration?

Food to Microorganism Ratio

10. An activated sludge aeration basin receives a primary effluent flow of 2.72 MGD with a BOD concentration of 198 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2610 mg/L and the aeration basin volume is 480,000 gallons. What is the current F/M ratio?
11. An activated sludge aeration basin receives a primary effluent flow of 3,350,000 gpd with a BOD of 148 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2510 mg/L and the aeration basin volume is 490,000 gallons. What is the F/M ratio?

12. The flow to a 195,000 gallon oxidation ditch is 320,000 gpd. The BOD concentration of the wastewater is 180 mg/L. If the mixed liquor suspended solids (MLSS) concentration is 2540 mg/L with a volatile solids content of 72%, what is the F/M ratio?
13. The desired F/M ratio at an extended aeration activated sludge plant is 0.7 lb BOD/lb MLVSS. If the primary effluent flow is 3.3 MGD and has a BOD of 181 mg/L, how many pounds of MLVSS should be maintained in the aeration basin?
14. The desired F/M ratio at a particular activated sludge plant is 0.4 lbs BOD/lb MLVSS. If the primary effluent flow is 2,510,000 gpd and has a BOD concentration of 141 mg/L, how many lbs of MLVSS should be maintained in the aeration basin?

Mean Cell Residence Time (MCRT), days

15. An activated sludge system has a total of 29,100 lbs of MLSS. The concentration of suspended solids leaving the final clarifier in the effluent is calculated to be 400 lbs/day. Suspended solids wasted from the clarifier are 2920 lbs/day. What is the MCRT in days?

16. Determine the MCRT given the following data: aeration basin volume, 1,500,000 gallons; mixed liquor suspended solids, 2710 mg/L; final clarifier, 106,000 gallons; waste activated sludge, 5870 mg/L; WAS pumping rate, 72,000 gpd; plant flow, 3.3 MGD; secondary effluent SS, 25 mg/L; average clarifier core SS, 1940 mg/L.
17. An aeration basin has a volume of 460,000 gallons. The final clarifier has a volume of 178,000 gallons. The MLSS concentration in the aeration basin is 2222 mg/L. If 1610 lbs/day suspended solids are wasted and 240 lbs/day suspended solids are in the secondary effluent, what is the MCRT for the activated sludge system?

18. Determine MCRT given the following information:

Aeration Basin = 350,000 gal
Final Clarifier = 125,000 gal
Flow = 1,400,000 gpd
WAS Pump Rate = 27,000 gpd

MLSS = 2910 mg/L
S.E. SS = 16 mg/L
WAS = 6210 mg/L

Wasting Rates

19. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.5 lbs BOD/lb MLVSS. It has been calculated that 3400 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 69%, how many lbs MLSS are desired in the aeration basin?

20. Using Constant MCRT: The desired MCRT for an activated sludge plant is 9 days. The secondary effluent flow is 3,220,000 gpd with a suspended solids content of 23 mg/L. There is a total of 32,400 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

21. Given the following data, determine the lbs/day suspended solids to be wasted:

Aeration Tank Volume = 1.2 MG
Influent Flow = 3,100,000 gpd
BOD = 110 mg/L

Desired F/M = 0.4
MLSS = 2200 mg/L
%VS = 68%

Answers:

- | | |
|---------------------|------------------------------|
| 1. 1761 lbs BOD/day | 12. 0.16 |
| 2. 5726 lbs COD/day | 13. 7116 lbs MLVSS |
| 3. 4459 lbs BOD/day | 14. 7379 lbs MLVSS |
| 4. 6105 lbs COD/day | 15. 8.8 days |
| 5. 8095 lbs MLSS | 16. 8.5 days |
| 6. 10,870 lbs MLVSS | 17. 6.4 days |
| 7. 7698 lbs MLVSS | 18. 7.3 days |
| 8. 9025 lbs MLSS | 19. 9855 lbs MLSS desired |
| 9. 9899 lbs MLVSS | 20. 2982 lbs MLSS to waste |
| 10. 0.43 | 21. 11,562 lbs MLSS to waste |
| 11. 0.40 | |

Applied Math for Wastewater Treatment Activated Sludge

BOD or COD Loading, lbs/day pg. 10 in formula book

1. The flow to an aeration tank is 850,000 gpd. If the BOD content of the wastewater entering the aeration tank is 225 mg/L, how many pounds of BOD are applied to the aeration tank daily?

$$\begin{aligned} \text{lbs BOD/day} &= (\text{BOD, mg/L})(\text{Flow, MGD})(8.34) \\ &= (225 \text{ mg/L})(0.85 \text{ MGD})(8.34) \\ &= \boxed{1595 \text{ lbs/d}} \end{aligned}$$

2. The flow to an aeration tank is 1200 gpm. If the COD concentration of the wastewater is 155 mg/L, what is the COD loading rate in lbs/day?

$$\begin{aligned} \frac{(1200 \text{ gpm})(1440)}{1,000,000} &= 1.728 \text{ MGD} \\ \text{lbs COD/day} &= (\text{COD, mg/L})(\text{Flow, MGD})(8.34) \\ &= (155 \text{ mg/L})(1.728 \text{ MGD})(8.34) \\ &= \boxed{2234 \text{ lbs/d}} \end{aligned}$$

Solids Inventory in the Aeration Tank, lbs. MLSS or lbs. MLVSS

3. An aeration basin is 120 ft long, 45 ft wide and holds wastewater to a depth of 12 ft. If the aeration basin has an MLSS concentration of 2150 mg/L, how many pounds of MLSS are under aeration?

$$\begin{aligned} \text{aerator volume, gal} &= (120 \text{ ft})(45 \text{ ft})(12 \text{ ft})(7.48) = 484,704 \text{ gal} \\ \text{MLSS, lbs} &= (\text{MLSS, mg/L})(\text{Aer. Vol, MG})(8.34) \\ &= (2150 \text{ mg/L})(0.484704 \text{ MG})(8.34) \\ &= \boxed{8691 \text{ lbs MLSS}} \end{aligned}$$

4. The aeration tank of a conventional activated sludge plant has an MLSS concentration of 2300 mg/L with a volatile solids content of 72%. If the volume of the aeration tank is 200,000 gallons, how many pounds of volatile solids are under aeration?

$$\begin{aligned} \text{MLVSS, lbs} &= (\text{MLSS, mg/L}) (\text{Aer. Vol., MG}) (8.34) (\% \text{ VS}) \\ &= (2300 \text{ mg/L}) (0.2 \text{ MG}) (8.34) (0.72) \\ &= \boxed{2762 \text{ lbs MLVSS}} \end{aligned}$$

Food to Microorganism Ratio

5. An activated sludge aeration tank receives a primary effluent flow of 1.6 MGD with a BOD concentration of 180 mg/L. The mixed liquor volatile suspended solids is 2200 mg/L and the aeration tank volume is 420,000 gallons. What is the current F/M ratio?

$$\begin{aligned} \text{F/M} &= \frac{(\text{BOD or COD, mg/L}) (\text{Flow, MGD}) (8.34)}{(\text{MLVSS, mg/L}) (\text{Aer. Vol., MG}) (8.34)} \\ &= \frac{(180 \text{ mg/L}) (1.6 \text{ MGD}) (8.34)}{(2200 \text{ mg/L}) (0.42 \text{ MG}) (8.34)} = \frac{2401.92}{7706.16} = \boxed{0.31} \end{aligned}$$

6. The flow to a 195,000 gallon oxidation ditch is 365,000 gpd. The BOD concentration of the wastewater is 170 mg/L. If the MLSS concentration is 2550 mg/L with a volatile content of 70%, what is the F/M ratio?

$$\begin{aligned} \text{F/M} &= \frac{(170 \text{ mg/L}) (0.365 \text{ MGD}) (8.34)}{(2550 \text{ mg/L}) (0.195 \text{ MG}) (8.34) (0.70)} \\ &= \frac{517.497}{2902.9455} = \boxed{0.18} \end{aligned}$$

7. The desired F/M ratio of an extended aeration activated sludge plant is 0.5 lbs COD/lb. MLVSS. If the 3.0 MGD primary effluent flow has a COD of 172 mg/L, how many lbs of MLVSS should be maintained in the aeration tank?

$$\begin{aligned} \text{Desired MLVSS, lbs} &= \frac{\text{BOD or COD, lbs}}{\text{Desired F/m ratio}} \\ &= \frac{(172 \text{ mg/L}) (3.0 \text{ MGD}) (8.34)}{0.5} \\ &= \boxed{8607 \text{ lbs}} \end{aligned}$$

Mean Cell Residence Time (MCRT), days pg. 11 formula book

8. An activated sludge system has a total of 28,500 lbs of mixed liquor suspended solids. The suspended solids leaving the final clarifier in the effluent is 400 lbs/day. The pounds suspended solids wasted from the final clarifier is 2910 lbs/day. What is the solids retention time (MCRT), days?

$$\begin{aligned} \text{MCRT} &= \frac{\text{SS in System, lbs}}{\text{WAS, lbs/d} + \text{SE SS lbs/d}} = \frac{28,500 \text{ lbs}}{2910 \text{ lbs/d} + 400 \text{ lbs/d}} \\ &= \frac{28,500 \text{ lbs}}{3310 \text{ lbs/d}} \\ &= \boxed{8.6 \text{ days}} \end{aligned}$$

9. Determine MCRT given the following information:

Aeration Tank = 1,400,000 gal

Final Clarifier = 105,000 gal

Flow = 3,000,000 gpd

WAS Pump Rate = 68,000 gpd

MLSS = 2650 mg/L

S.E. SS = 22 mg/L

CCSS = 1890 mg/L

WAS = 6050 mg/L

$$\begin{aligned} \text{MCRT, days} &= \frac{(\text{MLSS, mg/L})(\text{Aer. Vol, MG})(8.34) + (\text{CCSS, mg/L})(\text{Final Clar., Vol})(8.34)}{(\text{WAS, SS mg/L})(\text{WAS Flow MGD})(8.34) + (\text{SE SS mg/L})(\text{Plant Flow})(8.34)} \\ &= \frac{(2650)(1.4)(8.34) + (1890)(0.105)(8.34)}{(6050)(0.068)(8.34) + (22)(3.0)(8.34)} \\ &= \frac{30941.4 + 1655.073}{3431.076 + 550.44} = \frac{32596.473}{3981.516} = \boxed{8.2 \text{ days}} \end{aligned}$$

Wasting Rates

10. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.6 lbs BOD/lb MLVSS. It has been calculated that 3300 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 68%, how many lbs MLSS are desired in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{3300 \text{ lbs BOD}}{0.6} = 5500 \text{ lbs}$$

$$\text{Desired MLSS, lbs} = \frac{5500 \text{ lbs MLVSS}}{0.68} = \boxed{8088 \text{ lbs}}$$

11. Using Constant MCRT: The desired MCRT for an activated sludge plant is 8.5 days. The secondary effluent flow is 3.16 MGD with a suspended solids content of 22 mg/L. There is a total of 32,100 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

$$\begin{aligned}
 \text{WAS, lbs/d} &= \frac{(\text{MLSS, mg/L})(\text{Aer. Vol, MG})(8.34)}{\text{MCRT, days}} - (\text{SE SS, mg/L})(\text{Plant Q})(8.34) \\
 &= \frac{32,100 \text{ lbs}}{8.5 \text{ days}} - (22 \text{ mg/L})(3.16 \text{ MGD})(8.34) \\
 &= 3776.4706 \text{ lbs/d} - 579.7968 \text{ lbs/d} \\
 &= \boxed{3197 \text{ lbs/d}}
 \end{aligned}$$

Answers:

- | | |
|---------------------|---------------------------|
| 1. 1595 lbs BOD/day | 7. 8607 lbs MLVSS |
| 2. 2234 lbs COD/day | 8. 8.6 days |
| 3. 8691 lbs MLSS | 9. 8.2 days |
| 4. 2762 lbs MLVSS | 10. 8088 lbs MLSS desired |
| 5. 0.31 | 11. 3197 lbs to waste |
| 6. 0.18 | |

Applied Math for Wastewater Treatment

Activated Sludge

Extra Problems

BOD or COD Loading, lbs/day

1. The flow to an aeration basin is 880,000 gpd. If the BOD content of the wastewater entering the aeration basin is 240 mg/L, what is the lbs/day BOD loading?

$$\begin{aligned} \text{lbs/d} &= (240 \text{ mg/L})(0.88 \text{ MGD})(8.34) \\ &= \boxed{1761 \text{ lbs/d}} \end{aligned}$$

2. The flow to the aeration basin is 2980 gpm. If the COD concentration of the wastewater is 160 mg/L, how many lbs of COD are applied to the aeration basin daily?

$$\frac{(2980)(1440)}{1,000,000} = 4.2912 \text{ MGD}$$

$$\text{lbs/d} = (160 \text{ mg/L})(4.2912 \text{ MGD})(8.34) = \boxed{5726 \text{ lbs/d}}$$

3. The BOD content of the wastewater entering an aeration basin is 165 mg/L. If the flow to the aeration basin is 3,240,000 gpd, what is the lbs/day BOD loading?

$$\begin{aligned} \text{lbs/d} &= (165 \text{ mg/L})(3.24 \text{ MGD})(8.34) \\ &= \boxed{4459 \text{ lbs/d}} \end{aligned}$$

4. The daily flow to an aeration basin is 4,880,000 gpd. If the COD concentration of the influent wastewater is 150 mg/L, how many lbs of COD are applied to the aeration basin daily?

$$\begin{aligned} \text{lbs/d} &= (150 \text{ mg/L})(4.88 \text{ MGD})(8.34) \\ &= \boxed{6105 \text{ lbs/d}} \end{aligned}$$

Solids Inventory in the Aeration Basin, lbs. MLSS or lbs. MLVSS

5. If the mixed liquor suspended solids concentration is 2110 mg/L and the aeration basin has a volume of 460,000 gallons, how many lbs of suspended solids are in the aeration basin?

$$\begin{aligned} \text{lbs} &= (2110 \text{ lbs}) (0.46 \text{ MG}) (8.34) \\ &= \boxed{8095 \text{ lbs}} \end{aligned}$$

6. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2420 mg/L. If the aeration basin is 90 ft long by 50 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

$$\text{vol.} = \frac{(90 \text{ ft})(50 \text{ ft})(16 \text{ ft})(7.48)}{1,000,000} = 0.53856 \text{ MG}$$

$$\text{lbs} = (2420 \text{ mg/L})(0.53856 \text{ MG})(8.34) = \boxed{10,870 \text{ lbs}}$$

7. The aeration basin of a conventional activated sludge plant has a mixed liquor volatile suspended solids (MLVSS) concentration of 2410 mg/L. If the aeration basin is 80 ft long by 40 ft wide and has wastewater to a depth of 16 ft, how many lbs of MLVSS are under aeration?

$$\text{vol.} = \frac{(80 \text{ ft})(40 \text{ ft})(16 \text{ ft})(7.48)}{1,000,000} = 0.382976 \text{ MG}$$

$$\text{lbs} = (2410 \text{ mg/L})(0.382976 \text{ MG})(8.34) = \boxed{7698 \text{ lbs}}$$

8. An aeration basin is 110 ft long, 30 ft wide and has wastewater to a depth of 16 ft. If the aeration basin of this conventional activated sludge plant has a mixed liquor suspended solids (MLSS) concentration of 2740 mg/L, how many lbs of MLSS are under aeration?

$$\text{vol.} = \frac{(110 \text{ ft})(30 \text{ ft})(16 \text{ ft})(7.48)}{1,000,000} = 0.394944 \text{ MG}$$

$$\text{lbs} = (2740 \text{ mg/L})(0.394944 \text{ MG})(8.34) = \boxed{9025 \text{ lbs}}$$

9. An aeration basin is 110 ft long, 50 ft wide and has wastewater to a depth of 16 ft. If the mixed liquor suspended solids (MLSS) concentration in the aeration basin is 2470 mg/L with a volatile solids content of 73%, how many lbs of MLVSS are under aeration?

$$\text{Vol} = \frac{(110 \text{ ft})(50 \text{ ft})(16 \text{ ft})(7.48)}{1,000,000} = 0.65824 \text{ MG}$$

$$\text{lbs} = \underbrace{(2470 \text{ mg/L})(0.73)}_{\text{gives you mg/L MLVSS}}(0.65824)(8.34) = \boxed{9899 \text{ lbs}}$$

Food to Microorganism Ratio

10. An activated sludge aeration basin receives a primary effluent flow of 2.72 MGD with a BOD concentration of 198 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2610 mg/L and the aeration basin volume is 480,000 gallons. What is the current F/M ratio?

$$\begin{aligned} F/M &= \frac{(198 \text{ mg/L})(2.72 \text{ MGD})(8.34)}{(2610 \text{ mg/L})(0.48 \text{ MG})(8.34)} \\ &= \frac{4491.5904 \text{ lbs/d BOD}}{10448.352 \text{ lbs MLVSS}} = \boxed{0.43} \end{aligned}$$

11. An activated sludge aeration basin receives a primary effluent flow of 3,350,000 gpd with a BOD of 148 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration is 2510 mg/L and the aeration basin volume is 490,000 gallons. What is the F/M ratio?

$$\begin{aligned} F/M &= \frac{(148 \text{ mg/L})(3.35 \text{ MGD})(8.34)}{(2510 \text{ mg/L})(0.49 \text{ MG})(8.34)} \\ &= \frac{4134.972 \text{ lbs/d BOD}}{10257.316 \text{ lbs MLVSS}} = \boxed{0.40} \end{aligned}$$

12. The flow to a 195,000 gallon oxidation ditch is 320,000 gpd. The BOD concentration of the wastewater is 180 mg/L. If the mixed liquor suspended solids (MLSS) concentration is 2540 mg/L with a volatile solids content of 72%, what is the F/M ratio?

$$F/M = \frac{(180 \text{ mg/L})(0.32 \text{ MGD})(8.34)}{(2540 \text{ mg/L})(0.72)(0.195 \text{ MG})(8.34)}$$

$$= \frac{480.384 \text{ lbs/d BOD}}{2974,17744 \text{ lbs MLVSS}} = \boxed{0.16}$$

13. The desired F/M ratio at an extended aeration activated sludge plant is 0.7 lb BOD/lb MLVSS. If the primary effluent flow is 3.3 MGD and has a BOD of 181 mg/L, how many pounds of MLVSS should be maintained in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{(181 \text{ mg/L})(3.3 \text{ MGD})(8.34)}{0.7}$$

$$= \boxed{7116 \text{ lbs MLVSS}}$$

14. The desired F/M ratio at a particular activated sludge plant is 0.4 lbs BOD/lb MLVSS. If the primary effluent flow is 2,510,000 gpd and has a BOD concentration of 141 mg/L, how many lbs of MLVSS should be maintained in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{(141 \text{ mg/L})(2.51 \text{ MGD})(8.34)}{0.4}$$

$$= \boxed{7379 \text{ MLVSS lbs}}$$

Mean Cell Residence Time (MCRT), days

15. An activated sludge system has a total of 29,100 lbs of MLSS ^{in system}. The concentration of suspended solids leaving the final clarifier in the effluent is calculated to be 400 lbs/day. Suspended solids wasted from the clarifier are 2920 lbs/day. What is the MCRT in days? _{leaving}

$$\text{MCRT, days} = \frac{29,100 \text{ lbs}}{400 \text{ lbs/d} + 2920 \text{ lbs/d}}$$

$$= \frac{29,100 \text{ lbs}}{3320 \text{ lbs/d}} = \boxed{8.8 \text{ days}}$$

16. Determine the MCRT given the following data: aeration basin volume, 1,500,000 gallons; mixed liquor suspended solids, 2710 mg/L; final clarifier, 106,000 gallons; waste activated sludge, 5870 mg/L; WAS pumping rate, 72,000 gpd; plant flow, 3.3 MGD; secondary effluent SS, 25 mg/L; average clarifier core SS, 1940 mg/L.

Aer. Basin = 1,500,000 gal	MLSS = 2710 mg/L
Clarifier = 106,000 gal	WAS = 5870 mg/L
WAS Pump = 72,000 gpd	SE SS = 25 mg/L
Plant Flow = 3.3 MGD	CC SS = 1940 mg/L

★ Use 5th MCRT Formula ★

$$\text{MCRT} = \frac{(2710)(1.5)(8.34) + (1940)(0.106)(8.34)}{(5870)(0.072)(8.34) + (25)(3.3)(8.34)}$$

$$= \frac{33,902.1 + 1715.0376}{3524.8176 + 688.05} = \frac{35617.1376}{4212.8676} = \boxed{8.5 \text{ days}}$$

17. An aeration basin has a volume of 460,000 gallons. The final clarifier has a volume of 178,000 gallons. The MLSS concentration in the aeration basin is 2222 mg/L. If 1610 lbs/day suspended solids are wasted and 240 lbs/day suspended solids are in the secondary effluent, what is the MCRT for the activated sludge system?

Aer. Basin = 460,000 gal	SS wasted = 1610 lbs/d
Clarifier = 178,000 gal	SE SS = 240 lbs/d
MLSS = 2222 mg/L	

★ Use top of 4th MCRT formula + bottom of 2nd formula ★

$$\text{MCRT, days} = \frac{(\text{MLSS, mg/L})(\text{Aer. Vol} + \text{Clarifier Vol, MG})(8.34)}{\text{WAS, lbs/d} + \text{SE SS lbs/d}}$$

$$= \frac{(2222 \text{ mg/L})(0.460 + 0.178 \text{ MG})(8.34)}{1610 + 240 \text{ lbs/d}}$$

$$= \frac{11823.08424 \text{ lbs}}{1850 \text{ lbs/d}} = \boxed{6.4 \text{ days}}$$

18. Determine MCRT given the following information:

Aeration Basin = 350,000 gal
 Final Clarifier = 125,000 gal
 Flow = 1,400,000 gpd
 WAS Pump Rate = 27,000 gpd

MLSS = 2910 mg/L
 S.E. SS = 16 mg/L
 WAS = 6210 mg/L

★ Use 4th MCRT formula ★

$$\text{MCRT} = \frac{(2910 \text{ mg/L}) \overbrace{(0.35 + 0.125 \text{ MG})}^{0.475} (8.34)}{(6210 \text{ mg/L})(0.027 \text{ MGD})(8.34) + (16 \text{ mg/L})(1.4 \text{ MGD})(8.34)}$$

$$= \frac{11527.965 \text{ lbs}}{1398.3678 + 186.816} = \frac{11527.965}{1585.1838} = \boxed{7.3 \text{ d}}$$

Wasting Rates

19. Using Constant F/M Ratio: The desired F/M ratio for an activated sludge system is 0.5 lbs BOD/lb MLVSS. It has been calculated that 3400 lbs of BOD enter the aeration basin daily. If the volatile solids content of the MLSS is 69%, how many lbs MLSS are desired in the aeration basin?

$$\text{Desired MLVSS, lbs} = \frac{3400 \text{ lbs BOD}}{0.5} = 6800 \text{ lbs}$$

$$\text{Desired MLSS, lbs} = \frac{6800 \text{ lbs}}{0.69} = \boxed{9855 \text{ lbs}}$$

20. Using Constant MCRT: The desired MCRT for an activated sludge plant is 9 days. The secondary effluent flow is 3,220,000 gpd with a suspended solids content of 23 mg/L. There is a total of 32,400 lbs SS in the system. How many lbs/day WAS SS must be wasted to maintain the desired MCRT?

$$\text{WAS, lbs/d} = \frac{32,400 \text{ lbs}}{9 \text{ days}} - (23 \text{ mg/L})(3.22 \text{ MGD})(8.34)$$

$$= 3600 \text{ lbs/d} - 617.6604 \text{ lbs/d}$$

$$= \boxed{2982 \text{ lbs/d}}$$

21. Given the following data, determine the lbs/day suspended solids to be wasted:

Aeration Tank Volume = 1.2 MG

Influent Flow = 3,100,000 gpd

BOD = 110 mg/L

Desired F/M = 0.4

MLSS = 2200 mg/L

%VS = 68%

$$\begin{aligned} \text{Desired MLVSS, lbs/d} &= \frac{(110 \text{ mg/L})(3.1 \text{ MG})(8.34)}{0.4} \\ &= 7109.85 \text{ lbs} \end{aligned}$$

$$\text{Desired MLSS,} = \frac{7109.85}{0.68} = 10455.66176 \text{ lbs}$$

$$\text{Actual MLSS} = (2200 \text{ mg/L})(1.2 \text{ MG})(8.34) = 22017.6 \text{ lbs}$$

$$\text{MLSS to Waste} = 22017.6 - 10455.6 = 11,562 \text{ lbs MLSS to waste}$$

Answers:

- | | |
|---------------------|---------------------------------------|
| 1. 1761 lbs BOD/day | 12. 0.16 |
| 2. 5726 lbs COD/day | 13. 7116 lbs MLVSS |
| 3. 4459 lbs BOD/day | 14. 7379 lbs MLVSS |
| 4. 6105 lbs COD/day | 15. 8.8 days |
| 5. 8095 lbs MLSS | 16. 8.5 days |
| 6. 10,870 lbs MLVSS | 17. 6.4 days |
| 7. 7698 lbs MLVSS | 18. 7.3 days |
| 8. 9025 lbs MLSS | 19. 9855 lbs MLSS desired |
| 9. 9899 lbs MLVSS | 20. 2982 lbs MLSS to waste |
| 10. 0.43 | 21. 3977 lbs MLSS to waste |
| 11. 0.40 | 11,562 |

